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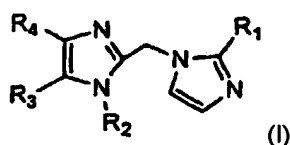
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(54) Title: SUBSTITUTED IMIDAZOLE DERIVATIVES: GABA<sub>A</sub> RECEPTOR LIGANDS



(I)

methods for treating such disorders are provided, as are methods for using such ligands for detecting GABA<sub>A</sub> receptors (e.g., receptor localization studies). (Formula I)

(57) Abstract: Substituted imidazole derivatives that bind to GABA<sub>A</sub> receptors are provided. Such compounds may be used to modulate ligand binding to GABA<sub>A</sub> receptors *in vivo* or *in vitro*, and are particularly useful in the treatment of a variety of central nervous system (CNS) disorders in humans, domesticated companion animals and livestock animals. Compounds provided herein may be administered alone or in combination with one or more other CNS agents to potentiate the effects of the other CNS agent(s). Pharmaceutical compositions and

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SUBSTITUTED IMIDAZOLE DERIVATIVES: GABA<sub>A</sub> RECEPTOR LIGANDSCROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Application 60/377,820, filed May 2, 2002 which is  
5 incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

This invention relates to substituted imidazole derivatives and specifically to imidazolyl methyl imidazole compounds. This invention also relates to pharmaceutical  
10 compositions comprising such compounds and to the use of such compounds in the treatment of central nervous system (CNS) diseases.

BACKGROUND OF THE INVENTION

The GABA<sub>A</sub> receptor superfamily represents one of the  
15 classes of receptors through which the major inhibitory neurotransmitter,  $\gamma$ -aminobutyric acid, or GABA, acts. Widely, although unequally, distributed throughout the mammalian brain, GABA mediates many of its actions through a complex of proteins called the GABA<sub>A</sub> receptor, which causes alteration in  
20 chloride conductance and membrane polarization. In addition to being the site of neurotransmitter action, a number of drugs including the anxiolytic and sedating benzodiazepines bind to this receptor. The GABA<sub>A</sub> receptor comprises a chloride channel that generally, but not invariably, opens in response to GABA,  
25 allowing chloride to enter the cell. This, in turn, effects a slowing of neuronal activity through hyperpolarization of the cell membrane potential.

GABA<sub>A</sub> receptors are composed of five protein subunits. A number of cDNAs for these GABA<sub>A</sub> receptor subunits have been  
30 cloned and their primary structures determined. While these subunits share a basic motif of 4 membrane-spanning helices, there is sufficient sequence diversity to classify them into

several groups. To date at least  $6\alpha$ ,  $3\beta$ ,  $3\gamma$ ,  $1\epsilon$ ,  $1\delta$  and  $2\rho$  subunits have been identified. Native GABA<sub>A</sub> receptors are typically composed of  $2\alpha$ ,  $2\beta$ , and  $1\gamma$  subunits (Pritchett & Seeburg *Science* 1989; 245:1389-1392, and Knight et. al., *Recept. Channels* 1998; 6:1-18). Various lines of evidence (such as message distribution, genome localization and biochemical study results) suggest that the major naturally occurring receptor combinations are  $\alpha_1\beta_2\gamma_2$ ,  $\alpha_2\beta_3\gamma_2$ ,  $\alpha_3\beta_3\gamma_2$ , and  $\alpha_5\beta_3\gamma_2$  (Mohler et al. *Neuroch. Res.* 1995; 20(5):631-36).

10 The GABA<sub>A</sub> receptor binding sites for GABA (2 per receptor complex) are formed by amino acids from the  $\alpha$  and  $\beta$  subunits. Amino acids from the  $\alpha$  and  $\gamma$  subunits together form one benzodiazepine site per receptor. Benzodiazepines exert their pharmacological actions by interacting with the benzodiazepine  
15 binding sites associated with the GABA<sub>A</sub> receptor. In addition to the benzodiazepine site (sometimes referred to as the benzodiazepine or BDZ receptor), the GABA<sub>A</sub> receptor contains sites of interaction for several other classes of drugs. These include a steroid binding site, a picrotoxin site, and a  
20 barbiturate site. The benzodiazepine site of the GABA<sub>A</sub> receptor is a distinct site on the receptor complex that does not overlap with the site of interaction for other classes of drugs that bind to the receptor or for GABA (see, e.g., Cooper, et al., *The Biochemical Basis of Neuropharmacology*, 6<sup>th</sup>  
25 ed., 1991, pp. 145-148, Oxford University Press, New York).

In a classic allosteric mechanism, the binding of a drug to the benzodiazepine site increases the affinity of the GABA receptor for GABA. Benzodiazepines and related drugs that enhance the ability of GABA to open GABA<sub>A</sub> receptor channels are  
30 known as agonists or partial agonists depending on the level of GABA enhancement. Other classes of drugs, such as  $\beta$ -carboline derivatives, that occupy the same site and negatively modulate the action of GABA are called inverse

agonists. A third class of compounds exists which occupy the same site as both the agonists and inverse agonists and yet have little or no effect on GABA activity. These compounds will, however, block the action of agonists or inverse agonists and are thus referred to as GABA<sub>A</sub> receptor antagonists.

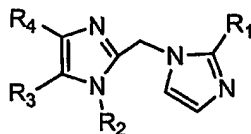
The important allosteric modulatory effects of drugs acting at the benzodiazepine site were recognized early, and the distribution of activities at different subtype receptors has been an area of intense pharmacological discovery. Agonists that act at the benzodiazepine site are known to exhibit anxiolytic, sedative, and hypnotic effects, while compounds that act as inverse agonists at this site elicit anxiogenic, cognition enhancing, and proconvulsant effects. While benzodiazepines have enjoyed long pharmaceutical use as anxiolytics, these compounds are known to exhibit a number of unwanted side effects. These may include cognitive impairment, sedation, ataxia, potentiation of ethanol effects, and a tendency for tolerance and drug dependence.

GABA<sub>A</sub> selective ligands may also act to potentiate the effects of certain other CNS active compounds. For example, there is evidence that selective serotonin reuptake inhibitors (SSRIs) may show greater antidepressant activity when used in combination with GABA<sub>A</sub> selective ligands than when used alone.

#### SUMMARY OF THE INVENTION

The present invention provides substituted imidazole derivatives that bind to GABA<sub>A</sub> receptors, including human GABA<sub>A</sub> receptors. Compounds provided herein act as agonists, antagonists or inverse agonists of such receptors, and are useful in the treatment of a variety of CNS disorders. Preferred compounds bind with high selectivity and/or high affinity to GABA<sub>A</sub> receptors.

In a broad aspect, the invention encompasses compounds represented by formula I and the pharmaceutically acceptable salts or prodrugs thereof.



Formula I

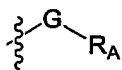
5 Within Formula I:

R<sub>1</sub> represents 5- to 10-membered aryl or heteroaryl, which is unsubstituted or substituted with from 1 to 4 groups independently selected from R<sub>5</sub>;

10 R<sub>2</sub> represents C<sub>1</sub>-C<sub>8</sub>alkyl, C<sub>2</sub>-C<sub>8</sub>alkenyl, C<sub>2</sub>-C<sub>8</sub>alkynyl, C<sub>3</sub>-C<sub>10</sub>cycloalkyl or (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)C<sub>1</sub>-C<sub>8</sub>alkyl, each of which is unsubstituted or substituted with from 1 to 3 substituents independently selected from R<sub>5</sub>;

R<sub>3</sub> and R<sub>4</sub> are each independently selected from:

- 15 (a) hydrogen, halogen, nitro and cyano; and  
(b) groups of the formula:



wherein (i) G is a bond, C<sub>1</sub>-C<sub>8</sub>alkylene, -NH-, -N(R<sub>B</sub>)-, -(R<sub>B</sub>)N-, -O-, -C(=O)-, -C(=O)NH-, -C(=O)NR<sub>B</sub>-, -S(O)<sub>m</sub>-, -CH<sub>2</sub>C(=O)-, -S(O)<sub>m</sub>NH-, -S(O)<sub>m</sub>NR<sub>B</sub>-, -NHC(=O)-, -C(=NR<sub>B</sub>)-, HC=N-, -NR<sub>B</sub>C(=O)-, -NHS(O)<sub>m</sub>- or -NR<sub>B</sub>S(O)-; and (ii) R<sub>A</sub> and R<sub>B</sub> are independently selected from C<sub>1</sub>-C<sub>8</sub>alkyl, C<sub>2</sub>-C<sub>8</sub>alkenyl, C<sub>2</sub>-C<sub>8</sub>alkynyl and 3- to 12-membered saturated, partially unsaturated and aromatic carbocycles and heterocycles having 1 ring or 2 fused, pendant or spiro rings, each of which is unsubstituted or substituted with from 1 to 4 substituents independently selected from R<sub>5</sub>; and (iii) m is 0, 1 or 2; and

20 R<sub>5</sub> is independently selected at each occurrence from halogen, hydroxy, nitro, cyano, amino, C<sub>1</sub>-C<sub>8</sub>alkyl, C<sub>1</sub>-C<sub>8</sub>alkoxy, mono- and di(C<sub>1</sub>-C<sub>8</sub>alkyl)amino, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)alkyl, (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)alkoxy, C<sub>2</sub>-

C<sub>9</sub>heterocycloalkyl, C<sub>1</sub>-C<sub>8</sub>alkenyl, C<sub>1</sub>-C<sub>8</sub>alkynyl, halo(C<sub>1</sub>-C<sub>8</sub>)alkyl, halo(C<sub>1</sub>-C<sub>8</sub>)alkoxy, oxo, amino(C<sub>1</sub>-C<sub>8</sub>)alkyl and mono- and di(C<sub>1</sub>-C<sub>8</sub>alkyl)amino(C<sub>1</sub>-C<sub>8</sub>)alkyl.

The present invention further provides pharmaceutical  
5 compositions comprising a compound as described above in  
combination with a physiologically acceptable carrier or  
excipient. Packaged pharmaceutical preparations are also  
provided, comprising such a pharmaceutical composition in a  
container and instructions for using the composition to treat  
10 a patient suffering from a CNS disorder such as anxiety,  
depression, a sleep disorder, attention deficit disorder or  
Alzheimer's dementia.

Methods are provided, within further aspects, for the  
treatment of patients suffering from certain CNS disorders  
15 (such as anxiety, depression, a sleep disorder, attention  
deficit disorder or Alzheimer's dementia), comprising  
administering to a patient in need of such treatment a  
therapeutically effective amount of a compound as described  
above. The patient may be a human or other mammal.  
20 Treatment of humans, domesticated companion animals (pets) or  
livestock animals suffering from certain CNS disorders with an  
effective amount of a compound of the invention is encompassed  
by the present invention.

Methods are also provided for improving short term memory  
25 in a patient, comprising administering to a patient in need of  
such treatment a therapeutically effective amount of a  
compound as described above. The patient may be a human or  
other mammal.

Within other aspects, the present invention provides  
30 methods for potentiating a therapeutic effect of a CNS agent,  
comprising administering to a patient a CNS agent and a  
compound as described above.

Methods for determining the presence or absence of GABA<sub>A</sub>  
receptor in a sample (e.g., a tissue section) are further

provided, comprising the steps of: (a) contacting a sample with a compound as described above under conditions that permit binding of the compound to GABA<sub>A</sub> receptor; and (b) detecting a level of compound bound to GABA<sub>A</sub> receptor.

5 The present invention further provides, within other aspects, methods for altering the signal-transducing activity of GABA<sub>A</sub> receptor, comprising contacting a cell expressing GABA<sub>A</sub> receptor with a compound as described above in an amount sufficient to detectably alter the electrophysiology of the  
10 cell.

These and other aspects of the present invention will become apparent upon reference to the following detailed description.

#### DETAILED DESCRIPTION OF THE INVENTION

15 As noted above, the present invention provides substituted imidazoles that bind to GABA<sub>A</sub> receptor, including human GABA<sub>A</sub> receptor. Without wishing to be bound to any particular theory, it is believed that the compounds provided herein bind to the benzodiazepine site of GABA<sub>A</sub> receptor, and  
20 that interaction of such compounds with the benzodiazepine site results in the pharmaceutical utility of these compounds. Compounds provided herein may be used in a variety of in vivo and in vitro contexts, as discussed in further detail below.

#### DEFINITIONS

25 Compounds of the present invention are generally described using standard nomenclature. Reference to a compound structure generally encompasses addition salts, hydrates and acylated prodrugs of the indicated structure, as well as all crystalline forms. The compounds herein  
30 described may have one or more asymmetric centers or planes. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present

invention. *Cis* and *trans* geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms. All chiral (enantiomeric and diastereomeric), and racemic forms, as well  
5 as all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomeric form is specifically indicated.

Certain compounds are described herein using a general formula that includes variables. Unless otherwise specified,  
10 each variable within such a formula is defined independently of other variable, and any variable that occurs more than one time within a formula is defined independently at each occurrence. Thus, for example, if a group is described as being substituted with 0-2 R\*, then the group may be  
15 unsubstituted or substituted with up to two R\* groups and R\* at each occurrence is selected independently from the definition of R\*. In addition, it will be apparent that combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

20 When any group, such as an aryl group, heteroaryl group, carbocycle or heterocycle, is said to be "substituted by one or more substituents" that group may contain from 1 to the maximum number of substituents allowable without exceeding the valency of the atoms of the substituted group. Preferably,  
25 such groups are substituted with from 1 to 4 substituents; more preferably, such groups are substituted with from 1 to 3 substituents. Such groups are further preferably substituted with zero or one oxo substituent. An "optionally substituted" group may be unsubstituted or substituted with from 1 to the  
30 maximum number of substituents indicated.

As used herein, "alkyl" refers to branched and straight-chain hydrocarbon groups. Preferred alkyl groups are C<sub>1</sub>-C<sub>8</sub>alkyl (i.e., alkyl groups having from 1 to 8 carbon atoms), with C<sub>1</sub>-C<sub>6</sub>alkyl and C<sub>1</sub>-C<sub>4</sub>alkyl particularly preferred. Examples



of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, isopentyl, s-pentyl, hexyl, 2-hexyl, 3-hexyl and 5-methylpentyl. An alkyl group may be bonded to an atom within  
5 a molecule of interest via any chemically suitable portion of the alkyl group.

The term "cycloalkyl" is intended to include saturated ring groups, having the specified number of carbon atoms, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or  
10 cycloheptyl. C<sub>3</sub>-C<sub>10</sub>cycloalkyl groups have from 3 to 10 ring members; preferred cycloalkyl groups have 4 to 8 and more preferably 5 to 7 ring members.

"Heterocycloalkyl" refers to saturated ring groups that comprise at least one heteroatom (i.e., N, S or O), with the  
15 remainder of the ring members carbon. Heterocycloalkyl groups typically include 3 to 10 rings members, preferably 4 to 8 and more preferably 5 to 7 ring members. Heterocycloalkyl groups typically have from 1 to 3 heteroatoms; preferably not more than one S atom and one O atom is present in a  
20 heterocycloalkyl group. Preferred heterocycloalkyl groups include morpholinyl, piperidinyl, piperazinyl, thiomorpholinyl, and pyrrolidinyl.

In the term "(cycloalkyl)alkyl" or (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)C<sub>1</sub>-C<sub>8</sub>alkyl, cycloalkyl and alkyl are as defined above and the  
25 point of attachment is on the alkyl group. This term encompasses, but is not limited to, cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl and cyclohexylmethyl. "(Heterocycloalkyl)alkyl" refers to such groups that comprise at least one heteroatom within the ring, as described above.

30 As used herein, "alkoxy" represents an alkyl group as defined above attached via an oxygen bridge. Preferred alkoxy groups have from 1 to 8 carbon atoms (i.e., C<sub>1</sub>-C<sub>8</sub>alkoxy). Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, 2-butoxy, t-butoxy,

n-pentoxy, 2-pentoxy, 3-pentoxy, isopentoxy, neopentoxy, n-hexoxy, 2-hexoxy, 3-hexoxy and 3-methylpentoxy. "C<sub>1</sub>-C<sub>6</sub>alkoxy" (alkoxy groups having from 1 to 6 carbon atoms) are preferred, with C<sub>1</sub>-C<sub>4</sub>alkoxy particularly preferred.

5 "Alkenyl" is intended to include hydrocarbon chains of either a straight or branched configuration comprising one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl and propenyl. Alkenyl groups typically will have 2 to about 8 carbon atoms,  
10 more typically 2 to about 6 carbon atoms. A "stable point" is bond that, when unsaturated, results in a chemically stable compound (i.e., a compound that can be isolated, characterized and tested for biological activity).

"Alkynyl" is intended to include hydrocarbon chains of  
15 either a straight or branched configuration comprising one or more triple carbon-carbon bonds which may occur in any stable point along the chain, such as ethynyl and propynyl. Alkynyl groups typically will have 2 to about 8 carbon atoms, more typically 2 to about 6 carbon atoms.

20 A "carbocycle" is a group that comprises at least one ring formed entirely by carbon-carbon bonds (referred to herein as a carbocyclic ring). Unless otherwise specified, such a ring may be aromatic or non-aromatic. A carbocycle generally has from 1 to 3 fused or pendant carbocyclic rings,  
25 preferably one ring or two fused carbocyclic rings. Typically, each ring contains from 3 to 8 (preferably from 5 to 7) ring members; carbocycles comprising fused or pendant ring systems typically contain from 9 to 12 ring members. Certain carbocycles are saturated cycloalkyl groups, as  
30 described above. Other carbocycles are "partially saturated" (i.e., comprise one or more double or triple bonds within a ring, but are not aromatic) or aryl groups (i.e., aromatic groups having 1 or more rings, wherein all members of the aromatic ring or rings are carbon). Preferred aryl groups

include 5- to 10-membered groups (i.e., single 5- to 7-membered rings or 7- to 10-membered bicyclic groups), such as phenyl and naphthyl. "Arylalkyl" groups (wherein aryl and alkyl are as defined above and the point of attachment is on the alkyl group) are also encompassed by the term "carbocycle." Such groups include, but are not limited to, benzyl. Carbon atoms present within a carbocycle ring may, of course, be further bonded to a variety of ring substituents, such as (but not limited to) hydrogen, a halogen, hydroxy, nitro, cyano, amino, C<sub>1</sub>-C<sub>8</sub>alkyl, C<sub>1</sub>-C<sub>8</sub>alkoxy, mono- and di(C<sub>1</sub>-C<sub>8</sub>alkyl)amino, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)alkyl, (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)alkoxy, C<sub>2</sub>-C<sub>9</sub>heterocycloalkyl, C<sub>1</sub>-C<sub>8</sub>alkenyl, C<sub>1</sub>-C<sub>8</sub>alkynyl, halo(C<sub>1</sub>-C<sub>8</sub>)alkyl, halo(C<sub>1</sub>-C<sub>8</sub>)alkoxy, oxo, amino(C<sub>1</sub>-C<sub>8</sub>)alkyl and mono- and di(C<sub>1</sub>-C<sub>8</sub>alkyl)amino(C<sub>1</sub>-C<sub>8</sub>)alkyl.

A "heterocycle" is a group that comprises at least one ring in which at least one ring atom is a heteroatom (i.e., N, O or S), and the remainder of the ring atoms are carbon. Such a ring is referred to as a heterocyclic ring. Preferably, a heterocyclic ring comprises 1-4 heteroatoms; within certain embodiments 1 or 2 heteroatoms is preferred. A heterocycle generally has from 1 to 3 fused or pendant rings (at least one of which is heterocyclic), preferably one ring or two fused rings. Typically, each ring contains from 3 to 8 ring members (preferably from 5 to 7 ring members); heterocycles comprising fused or pendant rings typically contain from 9 to 12 ring members. 3- to 10-membered heterocyclic groups that contain 1 heterocyclic ring or 2 fused rings (at least one of which is heterocyclic; for a total of 3 to 10 ring members) are preferred, with 5- to 10-membered heterocyclic groups particularly preferred. Heterocycles may be optionally substituted with one or more substituents as described above for carbocycles. Unless otherwise specified, a heterocycle may be saturated (i.e., heterocycloalkyl, as described above), partially saturated or aromatic (heteroaryl). As used herein

the term "heteroaryl" is intended to mean a stable 5-to 7-membered monocyclic and 7-to 10-membered bicyclic heterocyclic aromatic ring which consists of carbon atoms and from 1 to 4 heteroatoms independently selected from the group consisting of N, O and S. It is preferred that the total number of S and O atoms in the heteroaryl group, i.e., in the ring system, is not more than 1. In the term "heteroarylalkyl," heteroaryl and alkyl are as defined above and the point of attachment to the parent system is on the alkyl group.

Examples of heteroaryl groups include, but are not limited to, pyrimidinyl, pyridyl, quinolinyl, benzothienyl, indolyl, pyridazinyl, pyrazinyl, isoindolyl, isoquinolyl, quinazolinyl, quinoxalinyl, phthalazinyl, imidazolyl, isoxazolyl, pyrazolyl, oxazolyl, thienyl, thiazolyl, indoliziny, indazolyl, benzothiazolyl, benzimidazolyl, benzofuranyl, benzoisoxolyl, dihydro-benzodioxinyl, furanyl, pyrrolyl, oxadiazolyl, thiadiazolyl, triazolyl, tetrazolyl, oxazolopyridinyl, imidazopyridinyl, isothiazolyl, naphthyridinyl, cinnolinyl, carbazolyl, beta-carbolinyl, isochroman, chromanonyl, chromanyl, tetrahydroisoquinolinyl, isoindolinyl, isobenzotetrahydrofuran, isobenzotetrahydrothienyl, isobenzothienyl, benzoxazolyl, pyridopyridinyl, benzotetrahydrofuran, benzotetrahydrothienyl, purinyl, benzodioxolyl, triazinyl, phenoxazinyl, phenothiazinyl, pteridinyl, benzothiazolyl, imidazopyridinyl, imidazothiazolyl, dihydrobenzisoxazinyl, benzisoxazinyl, benzoxazinyl, dihydrobenzisothiazinyl, benzopyranyl, benzothiopyranyl, coumarinyl, isocoumarinyl, chromanyl, tetrahydroquinolinyl, dihydroquinolinyl, dihydroquinolinonyl, dihydroisoquinolinonyl, dihydrocoumarinyl, dihydroisocoumarinyl, isoindolinonyl, benzodioxanyl, benzoxazolinonyl, pyrrolyl N-oxide, pyrimidinyl N-oxide, pyridazinyl N-oxide, pyrazinyl N-oxide, quinolinyl N-oxide, indolyl N-oxide, indolinyl N-oxide, isoquinolyl N-

oxide, quinazolinyl N-oxide, quinoxalinyl N-oxide, phthalazinyl N-oxide, imidazolyl N-oxide, isoxazolyl N-oxide, oxazolyl N-oxide, thiazolyl N-oxide, indoliziny N-oxide, indazolyl N-oxide, benzothiazolyl N-oxide, benzimidazolyl N-oxide, pyrrolyl N-oxide, oxadiazolyl N-oxide, thiadiazolyl N-oxide, triazolyl N-oxide, tetrazolyl N-oxide, benzothiopyranyl S-oxide and benzothiopyranyl S,S-dioxide. Preferred heteroaryl groups include imidazolyl, pyrrolyl, pyridyl, thiazolyl, pyrazolyl, thiazolyl, isoxazolyl, triazolyl, tetrazolyl, oxadiazolyl, pyrimidinyl and oxazolyl, with pyridyl particularly preferred.

The term "halogen" includes fluorine, chlorine, bromine and iodine.

As used herein, "haloalkyl" refers to alkyl groups that are substituted with 1 or more halogen (for example  $-C_vF_w$  where v is an integer of from 1 to 3 and w is an integer of from 1 to  $(2v+1)$ ). Examples of haloalkyl groups include, but are not limited to, mono-, di- and tri-fluoromethyl; mono-, di- and tri-chloromethyl; mono-, di-, tri-, tetra- and penta-fluoroethyl; and mono-, di-, tri-, tetra- and penta-chloroethyl. "Halo( $C_1-C_8$ )alkyl" groups have 1 to 8 carbon atoms.

The term "haloalkoxy" refers to a haloalkyl group as defined above attached via an oxygen bridge. "Halo( $C_1-C_8$ )alkoxy" groups have 1 to 8 carbon atoms. Examples of haloalkoxy groups include, but are not limited to, mono-, di- and tri-fluoromethoxy.

The term "oxo," as used herein, refers to a keto ( $C=O$ ) group. An oxo group that is a substituent of a nonaromatic ring results in a conversion of  $-CH_2-$  to  $-C(=O)-$ . It will be apparent that the introduction of an oxo substituent on an aromatic ring destroys the aromaticity.

A "substituent," as used herein, refers to a molecular moiety that is covalently bonded to an atom within a molecule

of interest. For example, a "ring substituent" may be a moiety such as a halogen, alkyl group, alkoxy group, haloalkyl group or other group as discussed herein that is covalently bonded to an atom (preferably a carbon or nitrogen atom) that is a ring member. The term "substitution" refers to replacing a hydrogen atom in a molecular structure with a substituent as described above, such that the valence on the designated atom is not exceeded, and such that a chemically stable compound (i.e., a compound that can be isolated, characterized, and tested for biological activity) results from the substitution. Representative substituents include, but are not limited to, halogen, hydroxy, nitro, cyano, amino, C<sub>1</sub>-C<sub>8</sub>alkyl, C<sub>1</sub>-C<sub>8</sub>alkoxy, mono- and di(C<sub>1</sub>-C<sub>8</sub>alkyl)amino, C<sub>3</sub>-C<sub>10</sub>cycloalkyl, (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)alkyl, (C<sub>3</sub>-C<sub>10</sub>cycloalkyl)alkoxy, C<sub>2</sub>-C<sub>9</sub>heterocycloalkyl, C<sub>1</sub>-C<sub>8</sub>alkenyl, C<sub>1</sub>-C<sub>8</sub>alkynyl, halo(C<sub>1</sub>-C<sub>8</sub>)alkyl, halo(C<sub>1</sub>-C<sub>8</sub>)alkoxy, oxo, amino(C<sub>1</sub>-C<sub>8</sub>)alkyl and mono- and di(C<sub>1</sub>-C<sub>8</sub>alkyl)amino(C<sub>1</sub>-C<sub>8</sub>)alkyl.

A dash ("-") that is not between two letters or symbols is used to indicate a point of attachment for a substituent. For example, -CONH<sub>2</sub> is attached through the carbon atom.

The term "GABA<sub>A</sub> receptor" refers to a protein complex that detectably binds GABA and mediates a dose dependent alteration in chloride conductance and membrane polarization. Receptors comprising naturally-occurring mammalian (especially human or rat) GABA<sub>A</sub> receptor subunits are generally preferred, although subunits may be modified provided that any modifications do not substantially inhibit the receptor's ability to bind GABA (i.e., at least 50% of the binding affinity of the receptor for GABA is retained). The binding affinity of a candidate GABA<sub>A</sub> receptor for GABA may be evaluated using a standard ligand binding assay as provided herein. It will be apparent that there are a variety of GABA<sub>A</sub> receptor subtypes that fall within the scope of the term "GABA<sub>A</sub> receptor." These subtypes include, but are not limited to,  $\alpha_2\beta_3\gamma_2$ ,  $\alpha_3\beta_3\gamma_2$ ,  $\alpha_5\beta_3\gamma_2$ , and  $\alpha_1\beta_2\gamma_2$ .

receptor subtypes. GABA<sub>A</sub> receptors may be obtained from a variety of sources, such as from preparations of rat cortex or from cells expressing cloned human GABA<sub>A</sub> receptors. Particular subtypes may be readily prepared using standard techniques  
5 (e.g., by introducing mRNA encoded the desired subunits into a host cell, as described herein).

A "prodrug" is a compound that may not fully satisfy the structural requirements of the compounds provided herein, but is modified *in vivo*, following administration to a patient, to  
10 produce an active compound of the present invention. For example, a prodrug may be an acylated derivative of a compound as provided herein. Prodrugs include compounds wherein hydroxy, amine or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to  
15 form a free hydroxyl, amino or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups within the compounds provided herein.

A "patient" is any individual treated with a compound  
20 provided herein. Patients include humans, as well as other animals such as companion animals and livestock. Patients may be afflicted with a CNS disorder, or may be free of such a condition (*i.e.*, treatment may be prophylactic).

A "CNS disorder" is a disease or condition of the central  
25 nervous system that is responsive to GABA<sub>A</sub> receptor modulation in the patient. Such disorders include anxiety disorders (e.g., panic disorder, obsessive compulsive disorder, agoraphobia, social phobia, specific phobia, dysthymia, adjustment disorders, separation anxiety, cyclothymia, and  
30 generalized anxiety disorder), stress disorders (e.g., post-traumatic stress disorder, anticipatory anxiety acute stress disorder and acute stress disorder), depressive disorders (e.g., depression, atypical depression, bipolar disorder and depressed phase of bipolar disorder), sleep disorders (e.g.,

primary insomnia, circadian rhythm sleep disorder, dyssomnia NOS, parasomnias including nightmare disorder, sleep terror disorder, sleep disorders secondary to depression, anxiety and/or other mental disorders and substance-induced sleep disorder), cognitive disorders (e.g., cognition impairment, mild cognitive impairment (MCI), age-related cognitive decline (ARCD), traumatic brain injury, Down's Syndrome, neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and stroke), AIDS-associated dementia, dementia associated with depression, anxiety or psychosis, attention deficit disorders (e.g., attention deficit disorder and attention deficit and hyperactivity disorder), convulsive disorders (e.g., epilepsy), benzodiazepine overdose and drug and alcohol addiction.

A "CNS agent" is any drug used to treat or prevent a CNS disorder. CNS agents include, for example: serotonin receptor (e.g., 5-HT<sub>1A</sub>) agonists and antagonists and selective serotonin reuptake inhibitors (SSRIs); neurokinin receptor antagonists; corticotropin releasing factor receptor (CRF<sub>1</sub>) antagonists; melatonin receptor agonists; nicotinic agonists; muscarinic agents; acetylcholinesterase inhibitors and dopamine receptor agonists.

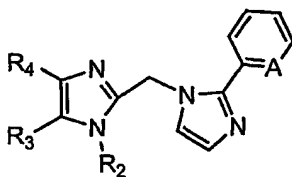
A compound is said to have "high affinity" if the K<sub>i</sub> at a GABA<sub>A</sub> receptor is less than 1 micromolar, preferably less than 100 nanomolar or less than 10 nanomolar. A representative assay for determining K<sub>i</sub> at GABA<sub>A</sub> receptor is provided in Example 3, herein. It will be apparent that the K<sub>i</sub> may depend upon the receptor subtype used in the assay. In other words, a high affinity compound may be "subtype-specific" (i.e., the K<sub>i</sub> is at least 10-fold greater for one subtype than for another subtype). Such compounds are said to have high affinity for GABA<sub>A</sub> receptor if the K<sub>i</sub> for at least one GABA<sub>A</sub> receptor subtype meets the above criteria.



A compound is said to have "high selectivity" if it binds to a GABA<sub>A</sub> receptor with a K<sub>i</sub> that is at least 10-fold lower, preferably at least 100-fold lower, than the K<sub>i</sub> for binding to other membrane-bound receptors. In particular, the compound  
5 should have a K<sub>i</sub> that is at least 10-fold greater at the following receptors than at a GABA<sub>A</sub> receptor: serotonin, dopamine, galanin, VR1, C5a, MCH, NPY, CRF, bradykinin, NK-1, NK-3 and tackykinin. Assays to determine the K<sub>i</sub> at other receptors may be performed using standard binding assay  
10 protocols.

Preferred compounds of Formula I are those in which R<sub>1</sub> is a 5- or 6-membered aromatic ring, unsubstituted or substituted with from 1 to 4 groups independently selected from R<sub>5</sub>. Representative preferred R<sub>1</sub> groups include phenyl and pyridyl,  
15 unsubstituted or substituted with from 1 to 3 groups independently selected from halogen, C<sub>1</sub>-C<sub>6</sub>alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, C<sub>1</sub>-C<sub>6</sub>alkoxy and halo(C<sub>1</sub>-C<sub>6</sub>)alkoxy. Preferred R<sub>1</sub> substituents include halogen, OH, C<sub>1</sub>-C<sub>6</sub>alkyl, and CF<sub>3</sub>. R<sub>1</sub> may be, for example, substituted with one or two halogens, such as  
20 fluorine.

Preferred compounds include those of Formula II, wherein A is CH or N, and other variable positions are as defined above:



Formula II

25 R<sub>2</sub> in Formulas I and II is preferably hydrogen, C<sub>1</sub>-C<sub>6</sub>alkyl or halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, more preferably C<sub>1</sub>-C<sub>4</sub>alkyl (e.g., ethyl or propyl).

R<sub>3</sub> and R<sub>4</sub> of Formulas I and II are preferably independently selected from hydrogen, halogen, alkyl, haloalkyl, and 5- to 7-membered aromatic carbocycles and  
30 heterocycles, wherein the carbocycles and heterocycles are

unsubstituted or substituted with halogen, trifluoromethyl or methyl. More preferably, R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, halogen, trifluoromethyl, methyl or phenyl, wherein the phenyl is unsubstituted or substituted with halogen, trifluoromethyl or methyl.

Preferred compounds of formula I and II also include compounds wherein R<sub>3</sub> is phenyl or pyridinyl and R<sub>4</sub> is hydrogen, trifluoromethyl or C<sub>1</sub>-C<sub>3</sub> alkyl, wherein phenyl and pyridinyl are optionally substituted with one of halogen, trifluoromethyl or methyl.

Preferred compounds of formula I and II also include compounds wherein R<sub>4</sub> is phenyl or pyridinyl and R<sub>3</sub> is hydrogen, trifluoromethyl, or C<sub>1</sub>-C<sub>3</sub> alkyl, wherein phenyl and pyridinyl are optionally substituted with one of halogen, trifluoromethyl or methyl.

Preferred compounds of formula I and II also include compounds wherein R<sub>3</sub> is C<sub>1</sub>-C<sub>3</sub> alkyl and R<sub>4</sub> is halogen, preferably bromine.

Preferred compounds of formula I and II also include compounds wherein R<sub>4</sub> is C<sub>1</sub>-C<sub>3</sub> alkyl and R<sub>3</sub> is halogen, preferably bromine.

Preferred compounds of formula I and II also include compounds wherein R<sub>3</sub> and R<sub>4</sub> are both hydrogen.

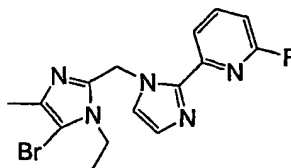
Preferred compounds of formula I and II also include compounds wherein R<sub>3</sub> and R<sub>4</sub> are both C<sub>1</sub>-C<sub>3</sub> alkyl.

Specific compounds provided herein include, but are not limited to:

Cmpd. No.

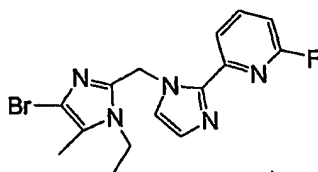
Compound

1



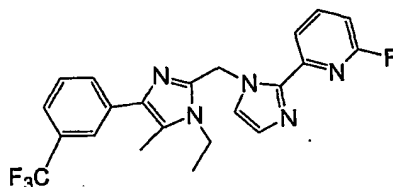
2-[1-(5-Bromo-1-ethyl-4-methyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoropyridine

2



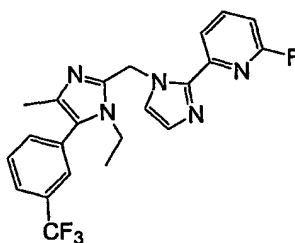
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3



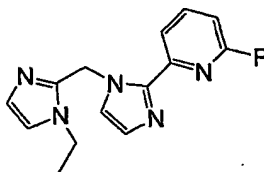
2-{1-[1-Ethyl-5-methyl-4-(3-trifluoromethyl-phenyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine

4



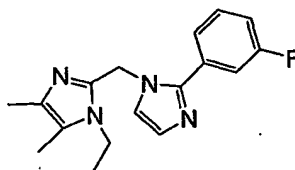
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5



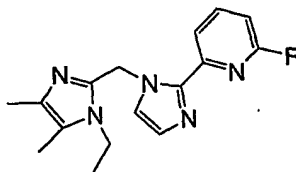
2-[1-(1-Ethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine

6



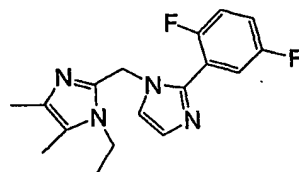
2-[1-(1-Ethyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-3-fluoro-benzene

7



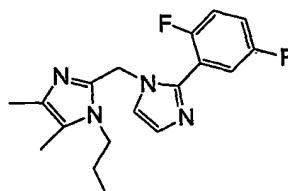
2-[1-(1-Ethyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-  
1H-imidazol-2-yl]-6-fluoro-pyridine

8



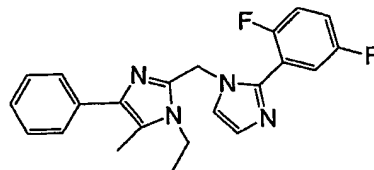
2-[1-(1-Ethyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-  
1H-imidazol-2-yl]-2,5-difluoro-benzene

9



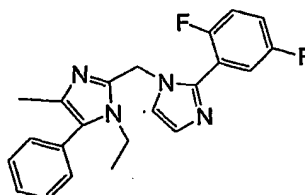
2-[1-(1-Propyl-4,5-dimethyl-1H-imidazol-2-  
ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene

10



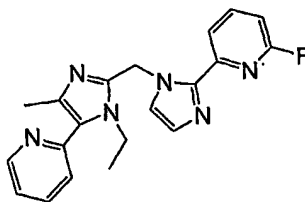
2-[1-(1-Ethyl-5-methyl-4-phenyl-1H-imidazol-2-  
ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene

11



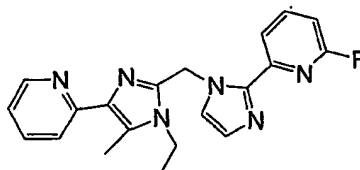
2-[1-(1-Ethyl-4-methyl-5-phenyl-1H-imidazol-2-  
ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene

12



2-{1-[1-ethyl-4-methyl-5-(2-pyridyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine

13



2-{1-[1-ethyl-5-methyl-4-(2-pyridyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine

It will be apparent that the specific compounds recited above are illustrative examples of compounds provided herein, and are not intended to limit the scope of the present invention. As noted above, all compounds of the present invention may be present as a free base or as a physiologically acceptable acid addition salt. In addition, both chiral compounds and racemic mixtures are encompassed by the present invention.

Compounds provided herein detectably alter (modulate) ligand binding to GABA<sub>A</sub> receptor, as determined using a standard *in vitro* receptor binding assay. References herein to a "GABA<sub>A</sub> receptor ligand binding assay" are intended to refer to the standard *in vitro* receptor binding assay provided in Example 3. Briefly, a competition assay may be performed in which a GABA<sub>A</sub> receptor preparation is incubated with labeled (e.g., <sup>3</sup>H) ligand, such as Flumazenil, and unlabeled test compound. Incubation with a compound that detectably modulates ligand binding to GABA<sub>A</sub> receptor will result in a decrease or increase in the amount of label bound to the GABA<sub>A</sub> receptor preparation, relative to the amount of label bound in the absence of the compound. Preferably, such a compound will

exhibit a  $K_i$  at GABA<sub>A</sub> receptor of less than 1 micromolar, more preferably less than 500 nM, 100 nM, 20 nM or 10 nM. The GABA<sub>A</sub> receptor used to determine *in vitro* binding may be obtained from a variety of sources, for example from preparations of  
5 rat cortex or from cells expressing cloned human GABA<sub>A</sub> receptors.

If desired, compounds provided herein may be evaluated for certain pharmacological properties including, but not limited to, solubility, oral bioavailability, toxicity, serum  
10 protein binding, lack of clinically relevant EKG effect and *in vitro* and *in vivo* half-life. Routine assays that are well known in the art may be used to assess these properties, and identify superior compounds for a particular use. For example, solubility in aqueous solutions is preferably at  
15 least 500 ng/mL. Assays used to predict bioavailability include transport across human intestinal cell monolayers, including Caco-2 cell monolayers. Toxicity may be assessed using any standard method, such as the assay detecting an effect on cellular ATP production provided in Example 5, or  
20 toxicity to cultured hepatocytes. Penetration of the blood brain barrier of a compound in humans may be predicted from the brain levels of the compound in laboratory animals given the compound intravenously. Serum protein binding may be predicted from albumin binding assays. Such assays are  
25 described in a review by Oravcová, et al. (Journal of Chromatography B (1996) volume 677, pages 1-27). Compound half-life is inversely proportional to the frequency of dosage of a compound. *In vitro* half-lives of compounds may be predicted from assays of microsomal half-life as described by  
30 Kuhnz and Gieschen (Drug Metabolism and Disposition, (1998) volume 26, pages 1120-1127).

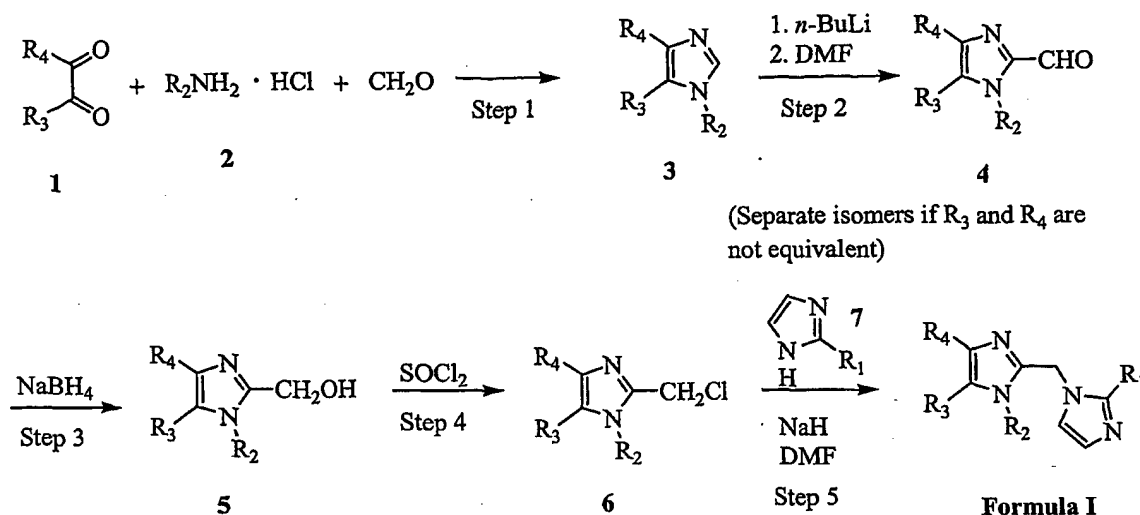
For detection purposes, as discussed in more detail below, compounds provided herein may be isotopically-labeled or radiolabeled. Such compounds are identical to those

described above, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into  
5 compounds provided herein include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as  $^2\text{H}$ ,  $^3\text{H}$ ,  $^{11}\text{C}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ,  $^{17}\text{O}$ ,  $^{31}\text{P}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{18}\text{F}$  and  $^{36}\text{Cl}$ . In addition, substitution with heavy isotopes such as deuterium (i.e.,  $^2\text{H}$ ) can afford certain therapeutic advantages  
10 resulting from greater metabolic stability, for example increased in vivo half-life or reduced dosage requirements and, hence, may be preferred in some circumstances.

#### PREPARATION OF COMPOUNDS

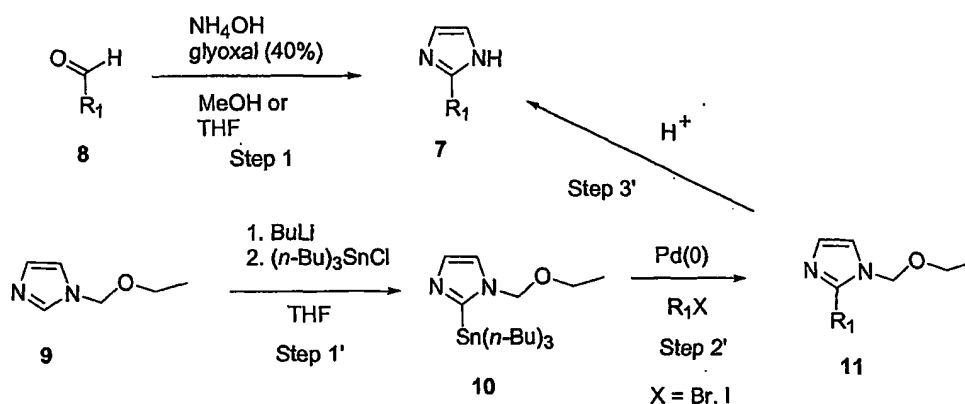
Compounds provided herein may generally be prepared  
15 using standard synthetic methods. Starting materials are generally readily available from commercial sources, such as Sigma-Aldrich Corp. (St. Louis, MO), or may be prepared as described herein. Representative procedures suitable for the preparation of compounds of Formula I are outlined in  
20 Schemes I-VII, herein, which are not to be construed as limiting the invention in scope or spirit to the specific reagents and conditions shown in them. Those having skill in the art will recognize that the reagents and conditions may be varied and additional steps employed to produce compounds  
25 encompassed by the present invention. In some cases, protection of reactive functionalities may be necessary to achieve the desired transformations. In general, such need for protecting groups, as well as the conditions necessary to attach and remove such groups, will be apparent to those  
30 skilled in the art of organic synthesis. Unless otherwise stated in the schemes below, the variables are as defined in Formula I.

## Scheme I

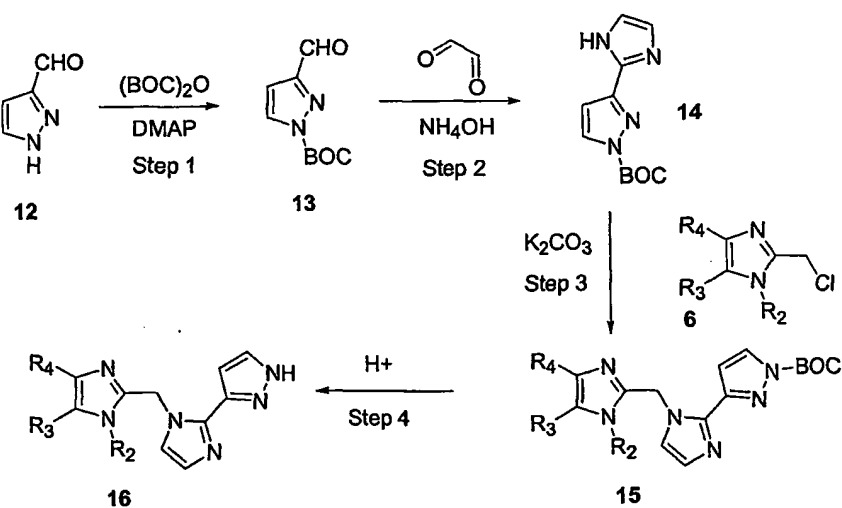


Scheme I illustrates a route to selected compounds of Formula I. In Step 1, diketone 1 are reacted with amine hydrochloride 2 and formaldehyde to obtain imidazole 3. When  $R_3$  and  $R_4$  are non-equivalent, imidazole 3 is obtained as an isomeric mixture. Subsequent reaction of 3 with *n*-butyl lithium in Step 2, followed by treatment with *N,N*-dimethylformamide provides aldehyde 4. When  $R_3$  and  $R_4$  are non-equivalent, the constituent isomers are often separated by chromatography on silica gel following Step 2. Optionally, when  $R_3$  or  $R_4$  is hydrogen, aldehyde 4 may be subjected to bromination, followed by palladium coupling chemistry to gain access to additional substituents at  $R_3$  at  $R_4$  (see Schemes IV and VI). In Steps 2 and 3, carboxaldehyde 4 is reduced to the corresponding alcohol 5 and subsequently converted (in Step 4) to chloride 6. In Step 5, the chloride 6 is reacted with the sodium salt of imidazole 7 to form a compound of Formula I. The synthesis of imidazole 7 is described in Scheme II, below.



Scheme II

- Scheme II illustrates two routes for the synthesis of an imidazole 7. Such compounds are intermediates in the synthesis of selected compounds of Formula I (see Scheme I).
- 5 In Step 1, an aryl or heteroaryl aldehyde 8 is treated with glyoxal and ammonium hydroxide to form imidazole 7. In Step 1', imidazole 9 is treated with butyl lithium followed by tri-*n*-butyltin chloride to obtain compound 10, which must be handled with care to avoid decomposition. In Step 2',
- 10 compound 10 is utilized in a palladium cross-coupling reaction with an aryl or heteroaryl halide to obtain compound 11. Subsequent treatment of 11 with acid in Step 3' provides compound 7.

Scheme III

Scheme III illustrates a route employing a protecting group strategy for preparing a pyrazole compound 16. In step 1, pyrazole 12 is reacted with di-tert-butylidicarbonate in the presence of 4-dimethylaminopyridine to obtain 13. Reaction  
5 with glyoxal and ammonium hydroxide provides 14. Reaction of 14 with chloromethyl compound 6 in the presence of base provides compound 15. Deprotection of 15 with acid in step 4 provides a pyrazole 16.

It will be apparent that the starting materials may be  
10 varied and additional steps employed to produce the compounds encompassed by the present invention.

In certain situations, compounds provided herein may contain one or more asymmetric carbon atoms, so that the compounds can exist in different stereoisomeric forms. These  
15 compounds can be, for example, racemates or optically active forms. As noted above, all stereoisomers are encompassed by the present invention. Nonetheless, it may be desirable to obtain single enantiomers (i.e., optically active forms). Standard methods for preparing single enantiomers include  
20 asymmetric synthesis and resolution of the racemates. Resolution of the racemates can be accomplished by conventional methods such as crystallization in the presence of a resolving agent, or chromatography using, for example, a chiral HPLC column.

25 As noted above, the present invention encompasses pharmaceutically acceptable salts of the compounds described herein. As used herein, a "pharmaceutically acceptable salt" is an acid or base salt that is generally considered in the art to be suitable for use in contact with the tissues of  
30 human beings or animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts,

including mineral and organic acid salts of basic residues such as amines, as well as alkali or organic salts of acidic residues such as carboxylic acids. Specific pharmaceutical salts include, but are not limited to, salts of acids such as

5 hydrochloric, phosphoric, hydrobromic, malic, glycolic, fumaric, sulfuric, sulfamic, sulfinic, sulfanilic, formic, toluenesulfonic, methanesulfonic, ethane disulfonic, 2-hydroxyethylsulfonic, oxalic, isethionic, nitric, benzoic, 2-acetoxybenzoic, citric, tartaric, lactic, stearic, salicylic,

10 glutamic, ascorbic, pamoic, succinic, fumaric, maleic, propionic, hydroxymaleic, hydroiodic, phenylacetic, alkanolic such as acetic,  $\text{HOOC}-(\text{CH}_2)_n-\text{COOH}$  where  $n$  is 0-4, and the like. Similarly, pharmaceutically acceptable cations include, but are not limited to sodium, potassium, calcium, aluminum,

15 lithium and ammonium. Those of ordinary skill in the art will recognize further pharmaceutically acceptable salts for the compounds provided herein, including those listed by *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, p. 1418 (1985). Accordingly, the present

20 disclosure should be construed to include all pharmaceutically acceptable salts of the compounds specifically recited.

A wide variety of synthetic procedures are available for the preparation of pharmaceutically acceptable salts. In general, a pharmaceutically acceptable salt can be synthesized

25 from a parent compound that contains a basic or acidic moiety by any conventional chemical method. Briefly, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of

30 the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred.

Prodrugs of the compounds provided herein may be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved to the parent

compounds. Prodrugs include compounds wherein hydroxy, amine or sulfhydryl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, or sulfhydryl group, respectively. Examples  
5 of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups within the compounds provided herein. Preferred prodrugs include acylated derivatives. Those of ordinary skill in the art will recognize various synthetic methods that  
10 may be employed to prepare prodrugs of the compounds provided herein.

Compounds may be radiolabeled by carrying out their synthesis using precursors comprising at least one atom that is a radioisotope. Such radioisotope(s) are preferably  
15 selected from carbon (preferably  $^{14}\text{C}$ ), hydrogen (preferably  $^3\text{H}$ ), sulfur (preferably  $^{35}\text{S}$ ), or iodine (preferably  $^{125}\text{I}$ ). Synthesis of such radiolabeled compounds may be conveniently performed by a radioisotope supplier specializing in custom synthesis of radiolabeled probe compounds, such as Amersham  
20 Corporation, Arlington Heights, IL; Cambridge Isotope Laboratories, Inc. Andover, MA; SRI International, Menlo Park, CA; Wizard Laboratories, West Sacramento, CA; ChemSyn Laboratories, Lexena, KS; American Radiolabeled Chemicals, Inc., St. Louis, MO; and Moravek Biochemicals Inc., Brea, CA.  
25 Tritium labeled compounds are also conveniently prepared catalytically via platinum-catalyzed exchange in tritiated acetic acid, acid-catalyzed exchange in tritiated trifluoroacetic acid, or heterogeneous-catalyzed exchange with tritium gas. Such preparations are also conveniently carried  
30 out as a custom radiolabeling by any of the suppliers listed above using the compound as substrate. In addition, certain precursors may be subjected to tritium-halogen exchange with tritium gas, tritium gas reduction of unsaturated bonds, or reduction using sodium borotritide, as appropriate.  $^{14}\text{C}$

radiolabeled compounds of the invention may be prepared using  $^{14}\text{C}$  radiolabeled diethyl oxalate (AMERICAN RADIOLABELED CHEMICALS, St. Louis, MO, catalog no. ARC-1127) as a starting material for the synthesis outlined in Scheme I.

## 5 PHARMACEUTICAL COMPOSITIONS

The present invention also provides pharmaceutical compositions comprising at least one compound provided herein, together with at least one physiologically acceptable carrier or excipient. Such compounds may be used for treating  
10 disorders responsive to GABA<sub>A</sub> receptor modulation (e.g., treatment of anxiety, depression, sleep disorders or cognitive impairment by GABA<sub>A</sub> receptor modulation). Pharmaceutical compositions may comprise, for example, water, buffers (e.g., neutral buffered saline or phosphate buffered saline),  
15 ethanol, mineral oil, vegetable oil, dimethylsulfoxide, carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, adjuvants, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione and/or preservatives. Preferred pharmaceutical  
20 compositions are formulated for oral delivery to humans or other animals (e.g., companion animals such as dogs). If desired, other active ingredients may also be included, such as CNS agents.

Pharmaceutical compositions may be formulated for any  
25 appropriate manner of administration, including, for example, topical, oral, nasal, rectal or parenteral administration. The term parenteral as used herein includes subcutaneous, intradermal, intravascular (e.g., intravenous), intramuscular, spinal, intracranial, intrathecal and intraperitoneal  
30 injection, as well as any similar injection or infusion technique. In certain embodiments, compositions in a form suitable for oral use are preferred. Such forms include, for example, tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard

or soft capsules, or syrups or elixirs. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate.

Compositions intended for oral use may further comprise one or more components such as sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide appealing and palatable preparations. Tablets contain the active ingredient in admixture with physiologically acceptable excipients that are suitable for the manufacture of tablets. Such excipients include, for example, inert diluents (e.g., calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate), granulating and disintegrating agents (e.g., corn starch or alginic acid), binding agents (e.g., starch, gelatin or acacia) and lubricating agents (e.g., magnesium stearate, stearic acid or talc). The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium (e.g., peanut oil, liquid paraffin or olive oil).

Aqueous suspensions comprise the active materials in admixture with one or more excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents (e.g., sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia); and dispersing or wetting agents (e.g., naturally-occurring phosphatides such as lecithin, condensation products of an

alkylene oxide with fatty acids such as polyoxyethylene stearate, condensation products of ethylene oxide with long chain aliphatic alcohols such as heptadecaethyleneoxycetanol, condensation products of ethylene oxide with partial esters  
5 derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides such as polyethylene sorbitan monooleate). Aqueous suspensions may also contain one or more preservatives, for  
10 example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil (e.g., arachis oil,  
15 olive oil, sesame oil or coconut oil) or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavoring agents may be added to provide palatable oral  
20 preparations. Such suspension may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting  
25 agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, such as sweetening, flavoring and coloring agents, may also be present.

30 Pharmaceutical compositions may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil (e.g., olive oil or arachis oil) or a mineral oil (e.g., liquid paraffin) or mixtures thereof. Suitable emulsifying agents may be naturally-occurring gums (e.g., gum acacia or

gum tragacanth), naturally-occurring phosphatides (e.g., soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol), anhydrides (e.g., sorbitan monoleate) and condensation products of partial esters derived  
5 from fatty acids and hexitol with ethylene oxide (e.g., polyoxyethylene sorbitan monoleate). The emulsions may also contain sweetening and/or flavoring agents..

Syrups and elixirs may be formulated with sweetening agents, such as glycerol, propylene glycol, sorbitol or  
10 sucrose. Such formulations may also comprise one or more demulcents, preservatives, flavoring agents and/or coloring agents.

A pharmaceutical composition may be prepared as a sterile injectible aqueous or oleaginous suspension. The compound,  
15 depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Such a composition may be formulated according to the known art using suitable dispersing, wetting agents and/or suspending agents such as those mentioned above. Among the acceptable vehicles and  
20 solvents that may be employed are water, 1,3-butanediol, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils may be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides. In  
25 addition, fatty acids such as oleic acid find use in the preparation of injectible compositions, and adjuvants such as local anesthetics, preservatives and/or buffering agents can be dissolved in the vehicle.

Pharmaceutical compositions may also be prepared in the  
30 form of suppositories (e.g., for rectal administration). Such compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable



excipients include, for example, cocoa butter and polyethylene glycols.

For administration to non-human animals, the composition may also be added to animal feed or drinking water. It may be  
5 convenient to formulate animal feed and drinking water compositions so that the animal takes in an appropriate quantity of the composition along with its diet. It may also be convenient to present the composition as a premix for addition to feed or drinking water.

10 Pharmaceutical compositions may be formulated as sustained release formulations (i.e., a formulation such as a capsule that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology and administered by, for example,  
15 oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active compound release. The amount of compound  
20 contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Compounds provided herein are generally present within a  
25 pharmaceutical composition in a therapeutically effective amount. A therapeutically effective amount is an amount that results in a discernible patient benefit, such as diminution of symptoms of a CNS disorder. A preferred concentration is one sufficient to inhibit the binding of GABA<sub>A</sub> receptor ligand  
30 to GABA<sub>A</sub> receptor *in vitro*. Compositions providing dosage levels ranging from about 0.1 mg to about 140 mg per kilogram of body weight per day are preferred (about 0.5 mg to about 7 g per human patient per day). The amount of active ingredient that may be combined with the carrier materials to

produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient. It will be understood, however, that the optimal dose for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time and route of administration; the rate of excretion; any simultaneous treatment, such as a drug combination; and the type and severity of the particular disease undergoing treatment. Optimal dosages may be established using routine testing, and procedures that are well known in the art.

Pharmaceutical compositions may be packaged for treating a CNS disorder such as anxiety, depression, a sleep disorder, attention deficit disorder or Alzheimer's dementia. Packaged pharmaceutical preparations include a container holding a therapeutically effective amount of at least one compound as described herein and instructions (e.g., labeling) indicating that the contained composition is to be used for treating the CNS disorder.

#### METHODS OF USE

Within certain aspects, the present invention provides methods for inhibiting the development of a CNS disorder. In other words, therapeutic methods provided herein may be used to treat a disorder, or may be used to prevent or delay the onset of such a disease in a patient who is free of detectable CNS disorder. CNS disorders are discussed in more detail below, and may be diagnosed and monitored using criteria that have been established in the art. Alternatively, or in addition, compounds provided herein may be administered to a patient to improve short-term memory. Patients may include humans, domesticated companion animals (pets, such as dogs)

and livestock animals, with dosages and treatment regimes as described above.

Frequency of dosage may vary, depending on the compound used and the particular disease to be treated or prevented.

5 In general, for treatment of most disorders, a dosage regimen of 4 times daily or less is preferred. For the treatment of sleep disorders a single dose that rapidly reaches effective concentrations is desirable. Patients may generally be monitored for therapeutic effectiveness using assays suitable  
10 for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

Within preferred embodiments, compounds provided herein are used to treat patients in need of such treatment, in an amount sufficient to alter the symptoms of a CNS disorder.

15 Compounds that act as agonists at  $\alpha_2\beta_3\gamma_2$  and  $\alpha_3\beta_3\gamma_2$  receptor subtypes are particularly useful in treating anxiety disorders such as panic disorder, obsessive compulsive disorder and generalized anxiety disorder; stress disorders including post-traumatic stress, and acute stress disorders. Compounds that  
20 act as agonists at  $\alpha_2\beta_3\gamma_2$  and  $\alpha_3\beta_3\gamma_2$  receptor subtypes are also useful in treating depressive or bipolar disorders and in treating sleep disorders. Compounds that act as inverse agonists at the  $\alpha_5\beta_3\gamma_2$  receptor subtype or  $\alpha_1\beta_2\gamma_2$  and  $\alpha_5\beta_3\gamma_2$  receptor subtypes are particularly useful in treating  
25 cognitive disorders including those resulting from Down's Syndrome, neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and stroke related dementia. Compounds of the invention that act as inverse agonists at the  $\alpha_5\beta_3\gamma_2$  are particularly useful in treating cognitive disorders  
30 through the enhancement of memory, and particularly short-term memory, in memory-impaired patients. Compounds that act as agonists at the  $\alpha_1\beta_2\gamma_2$  receptor subtype are useful in treating convulsive disorders such as epilepsy. Compounds that act as

antagonists at the benzodiazepine site are useful in reversing the effect of benzodiazepine overdose and in treating drug and alcohol addiction.

5 CNS disorders that can be treated using compounds and compositions provided herein include:

Depression, e.g., depression, atypical depression, bipolar disorder, depressed phase of bipolar disorder.

10 Anxiety, e.g., general anxiety disorder (GAD), agoraphobia, panic disorder +/- agoraphobia, social phobia, specific phobia, Post traumatic stress disorder, obsessive compulsive disorder (OCD), dysthymia, adjustment disorders with disturbance of mood and anxiety, separation anxiety disorder, anticipatory anxiety acute stress disorder, adjustment disorders, cyclothymia.

15 Sleep disorders, e.g., sleep disorders including primary insomnia, circadian rhythm sleep disorder, dyssomnia NOS, parasomnias, including nightmare disorder, sleep terror disorder, sleep disorders secondary to depression and/or anxiety or other mental disorders, substance induced sleep disorder.

20 Cognition Impairment, e.g., cognition impairment, Alzheimer's disease, Parkinson's disease, mild cognitive impairment (MCI), age-related cognitive decline (ARCD), stroke, traumatic brain injury, AIDS associated dementia, and dementia associated with depression, anxiety and psychosis (including schizophrenia and hallucinatory disorders).

25 Attention Deficit Disorder, e.g., attention deficit disorder (ADD), and attention deficit and hyperactivity disorder (ADHD).

30 Speech disorders, e.g., motor tic, clonic stuttering, dysfluency, speech blockage, dysarthria, Tourette's Syndrome and logospasm.

Compounds and compositions provided herein can also be used to improve short-term memory (working memory) in a patient. A therapeutically effective amount of a compound for improving short-term memory loss is an amount sufficient to  
5 result in a statistically significant improvement in any standard test of short-term memory function, including forward digit span and serial rote learning. For example, such a test may be designed to evaluate the ability of a patient to recall words or letters. Alternatively, a more complete  
10 neurophysical evaluation may be used to assess short-term memory function. Patients treated in order to improve short-term memory may, but need not, have been diagnosed with memory impairment or considered predisposed to development of such impairment.

15 In a separate aspect, the present invention provides methods for potentiating the action (or therapeutic effect) of other CNS agent(s). Such methods comprise administering an effective amount of a compound provided herein in combination with another CNS agent. CNS agents include, but are not  
20 limited to the following: for anxiety, serotonin receptor (e.g., 5-HT<sub>1A</sub>) agonists and antagonists; for anxiety and depression, neurokinin receptor antagonists or corticotropin releasing factor receptor (CRF<sub>1</sub>) antagonists; for sleep disorders, melatonin receptor agonists; and for  
25 neurodegenerative disorders, such as Alzheimer's dementia, nicotinic agonists, muscarinic agents, acetylcholinesterase inhibitors and dopamine receptor agonists. Within preferred embodiments, the present invention provides a method of potentiating the antidepressant activity of selective  
30 serotonin reuptake inhibitors (SSRIs) by administering an effective amount of a GABA agonist compound of the invention in combination with an SSRI. An effective amount of compound is an amount sufficient to result in a detectable change in

patient symptoms, when compared to a patient treated with the other CNS agent alone.

Combination administration can be carried out in a fashion analogous to that disclosed in Da-Rocha, et al., *J. Psychopharmacology* (1997) 11(3):211-218; Smith, et al., *Am. J. Psychiatry* (1998) 155(10):1339-45; or Le, et al., *Alcohol and Alcoholism* (1996) 31(suppl.):127-132. See also PCT International Publication Nos. WO 99/47142; WO 99/47171; WO 99/47131; and WO 99/37303.

10 The present invention also pertains to methods of inhibiting the binding of benzodiazepine compounds (i.e., compounds that comprise the benzodiazepine ring structure), such as Ro15-1788 or GABA, to the GABA<sub>A</sub> receptors. Such methods involve contacting a compound provided herein with  
15 cells expressing GABA<sub>A</sub> receptor, wherein the compound is present in an amount sufficient to inhibit benzodiazepine binding or GABA binding to GABA<sub>A</sub> receptors *in vitro*. This method includes inhibiting the binding of benzodiazepine compounds to GABA<sub>A</sub> receptors *in vivo* (e.g., in a patient given  
20 an amount of a compound provided herein that would be sufficient to inhibit the binding of benzodiazepine compounds or GABA to GABA<sub>A</sub> receptors *in vitro*). In one embodiment, such methods are useful in treating benzodiazepine drug overdose. The amount of a compound that would be sufficient to inhibit  
25 the binding of a benzodiazepine compound to the GABA<sub>A</sub> receptor may be readily determined via an GABA<sub>A</sub> receptor binding assay, such as the assay described in Example 3.

Within separate aspects, the present invention provides a variety of *in vitro* uses for the compounds provided herein.  
30 For example, such compounds may be used as probes for the detection and localization of GABA<sub>A</sub> receptors, in samples such as tissue sections, as positive controls in assays for receptor activity, as standards and reagents for determining the ability of a candidate agent to bind to GABA<sub>A</sub> receptor, or

as radiotracers for positron emission tomography (PET) imaging or for single photon emission computerized tomography (SPECT). Such assays can be used to characterize GABA<sub>A</sub> receptors in living subjects. Such compounds are also useful as standards  
5 and reagents in determining the ability of a potential pharmaceutical to bind to GABA<sub>A</sub> receptor.

Within methods for determining the presence or absence of GABA<sub>A</sub> receptor in a sample, a sample may be incubated with a compound as provided herein under conditions that permit  
10 binding of the compound to GABA<sub>A</sub> receptor. The amount of compound bound to GABA<sub>A</sub> receptor in the sample is then detected. For example, a compound may be labeled using any of a variety of well known techniques (e.g., radiolabeled with a radionuclide such as tritium, as described herein), and  
15 incubated with the sample (which may be, for example, a preparation of cultured cells, a tissue preparation or a fraction thereof). A suitable incubation time may generally be determined by assaying the level of binding that occurs over a period of time. Following incubation, unbound compound  
20 is removed, and bound compound detected using any method for the label employed (e.g., autoradiography or scintillation counting for radiolabeled compounds; spectroscopic methods may be used to detect luminescent groups and fluorescent groups). As a control, a matched sample may be simultaneously contacted  
25 with radiolabeled compound and a greater amount of unlabeled compound. Unbound labeled and unlabeled compound is then removed in the same fashion, and bound label is detected. A greater amount of detectable label in the test sample than in the control indicates the presence of capsaicin receptor in  
30 the sample. Detection assays, including receptor autoradiography (receptor mapping) of GABA<sub>A</sub> receptors in cultured cells or tissue samples may be performed as described by Kuhar in sections 8.1.1 to 8.1.9 of Current Protocols in Pharmacology (1998) John Wiley & Sons, New York.

For example, compounds provided herein may be used for detecting GABA<sub>A</sub> receptors in cell or tissue samples. This may be done by preparing a plurality of matched cell or tissue samples, at least one of which is prepared as an experimental sample and at least one of which is prepared as a control sample. The experimental sample is prepared by contacting (under conditions that permit binding of RO15-1788 to GABA<sub>A</sub> receptors within cell and tissue samples) at least one of the matched cell or tissue samples that has not previously been contacted with any compound provided herein with an experimental solution comprising a detectably-labeled preparation of the selected compound at the first measured molar concentration. The control sample is prepared in the same manner as the experimental sample and also contains an unlabelled preparation of the same compound at a greater molar concentration.

The experimental and control samples are then washed to remove unbound detectably-labeled compound. The amount of remaining bound detectably-labeled compound is then measured and the amount of detectably-labeled compound in the experimental and control samples is compared. A comparison that indicates the detection of a greater amount of detectable label in the at least one washed experimental sample than is detected in any of control samples demonstrates the presence of GABA<sub>A</sub> receptor in the experimental sample.

The detectably-labeled compound used in this procedure may be labeled with a radioactive label or a directly or indirectly luminescent label. When tissue sections are used in this procedure and the detectably-labeled compound is radiolabeled, the bound, labeled compound may be detected autoradiographically to generate an autoradiogram. The amount of detectable label in an experimental or control sample may be measured by viewing the autoradiograms and comparing the exposure density of the autoradiograms.



Compounds provided herein may also be used within a variety of well known cell culture and cell separation methods. For example, compounds may be linked to the interior surface of a tissue culture plate or other cell culture support, for use in immobilizing GABA<sub>A</sub> receptor-expressing cells for screens, assays and growth in culture. Such linkage may be performed by any suitable technique, such as the methods described above, as well as other standard techniques. Compounds may also be used to facilitate cell identification and sorting *in vitro*, permitting the selection of cells expressing a GABA<sub>A</sub> receptor. Preferably, the compound(s) for use in such methods are labeled as described herein. Within one preferred embodiment, a compound linked to a fluorescent marker, such as fluorescein, is contacted with the cells, which are then analyzed by fluorescence activated cell sorting (FACS).

Within other aspects, methods are provided for modulating binding of ligand to a GABA<sub>A</sub> receptor *in vitro* or *in vivo*, comprising contacting a GABA<sub>A</sub> receptor with a sufficient amount of a compound provided herein, under conditions suitable for binding of ligand to the receptor. The GABA<sub>A</sub> receptor may be present in solution, in a cultured or isolated cell preparation or within a patient. Preferably, the GABA<sub>A</sub> receptor is present in the brain of a mammal. In general, the amount of compound contacted with the receptor should be sufficient to modulate ligand binding to GABA<sub>A</sub> receptor *in vitro* within, for example, a binding assay as described in Example 3.

Also provided herein are methods for altering the signal-transducing activity of cellular GABA<sub>A</sub> receptor (particularly the chloride ion conductance), by contacting GABA<sub>A</sub> receptor, either *in vitro* or *in vivo*, with a sufficient amount of a compound as described above, under conditions suitable for binding of ligand to the receptor. The GABA<sub>A</sub> receptor may be

present in solution, in a cultured or isolated cell preparation or within a patient, and the amount of compound may be an amount that would be sufficient to alter the signal-transducing activity of GABA<sub>A</sub> receptors *in vitro*. In general, the amount of compound contacted with the receptor should be sufficient to modulate ligand binding to GABA<sub>A</sub> receptor *in vitro* within, for example, a binding assay as described in Example 3. An effect on signal-transducing activity may be assessed as an alteration in the electrophysiology of the cells, using standard techniques. If the receptor is present in an animal, an alteration in the electrophysiology of the cell may be detected as a change in the animal's feeding behavior. The amount of a compound that would be sufficient to alter the signal-transducing activity of GABA<sub>A</sub> receptors may be determined via a GABA<sub>A</sub> receptor signal transduction assay, such as the assay described in Example 4. The cells expressing the GABA receptors *in vivo* may be, but are not limited to, neuronal cells or brain cells. Such cells may be contacted with compounds of the invention through contact with a body fluid containing the compound, for example through contact with cerebrospinal fluid. Alteration of the signal-transducing activity of GABA<sub>A</sub> receptors *in vitro* may be determined from a detectable change in the electrophysiology of cells expressing GABA<sub>A</sub> receptors, when such cells are contacted with a compound of the invention in the presence of GABA.

Intracellular recording or patch-clamp recording may be used to quantitate changes in electrophysiology of cells. A reproducible change in behavior of an animal given a compound of the invention may also be used to indicate that a change in the electrophysiology of the animal's cells expressing GABA<sub>A</sub> receptors has occurred.

The following Examples are offered by way of illustration and not by way of limitation. Unless otherwise specified, all

reagents and solvents are of standard commercial grade and are used without further purification. Starting materials and intermediates described herein may generally be obtained from commercial sources, prepared from commercially available organic compounds or prepared using well known synthetic methods.

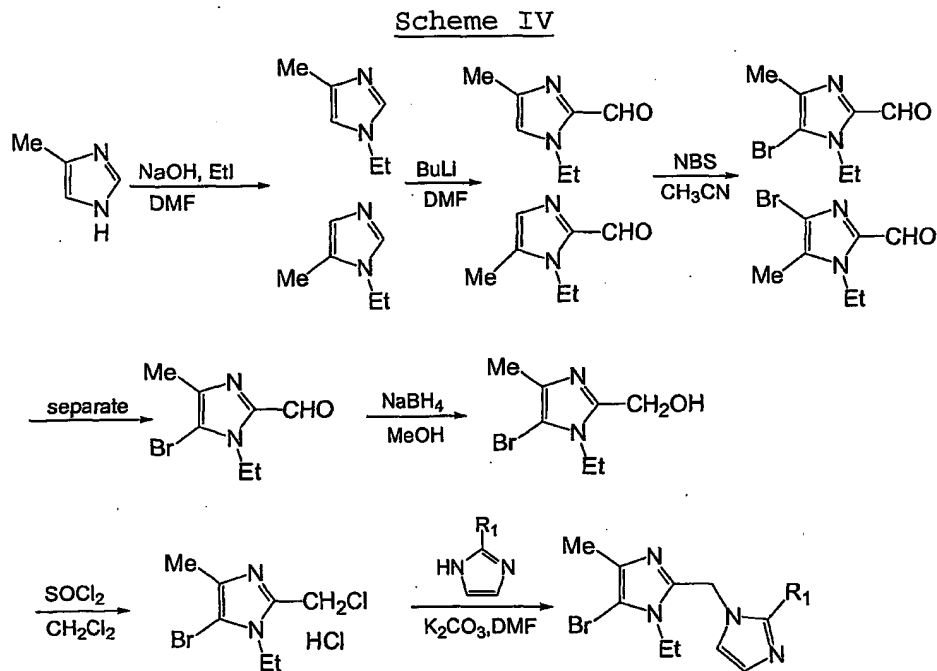
## EXAMPLES

### EXAMPLE 1

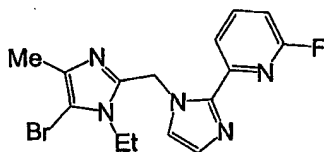
#### 10 Preparation of Methyl-Bromo-Imidazoles and Related Compounds

This Example illustrates the preparation of representative methyl-bromo-imidazole compounds and related compounds provided herein.

Methyl-bromo-imidazole compounds are prepared according to the following general scheme:



20 A. 2-[1-(5-BROMO-1-ETHYL-4-METHYL-1H-IMIDAZOL-2-YLMETHYL)-1H-IMIDAZOL-2-YL]-6-FLUORO-PYRIDINE



1. 1-Ethyl-4-methyl-1H-imidazole and 1-ethyl-5-methyl-1H-imidazole

To a solution of 3.28 g (40 mmol) 4-methyl imidazole in  
5 10 mL DMF chilled to 0°C is added 1.76 g (44 mmol) finely  
grounded NaOH. The suspension is stirred for 10 minutes and  
3.52 ml (44 mmol) iodoethane was added. The suspension is  
stirred at 0°C for 2 hours, and then at room temperature for  
24 hours. The solvent is removed in vacuo and the residue is  
10 partitioned with 8 ml of water and 15 ml of EtOAc. The  
aqueous layer is extracted with EtOAc (2 x 15 ml) and the  
combined extracts are washed with 10 ml of brine, then dried  
(Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated in vacuo. This clear oil is  
used in next step without further purification. MS m/z (M +  
15 1)<sup>+</sup> 111.

2. 1-Ethyl-4-methyl-1H-imidazole-2-carbaldehyde and 1-ethyl-5-methyl-1H-imidazole-2-carbaldehyde

To a solution of 2.22 g (20 mmol) 1-ethyl-4-methyl-1H-  
imidazole-2-carbaldehyde and 1-ethyl-5-methyl-1H-imidazole-2-  
20 carbaldehyde in 30 ml THF chilled to -78°C is added 10 ml BuLi  
(2.5 M in hexane, 25 mmol). The mixture is stirred at -78°C  
for 30 minutes, and then DMF (3 ml) is added. The cooling  
bath is removed and the mixture is stirred at room temperature  
for 24 hours. The solvent is removed in vacuo and the residue  
25 is partitioned with 10 ml of water and 25 ml of EtOAc. The  
aqueous layer is extracted with EtOAc (2 x 25 ml) and the  
combined extracts are washed with 20 ml of brine, and then  
dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated in vacuo. The residue  
is purified by flash column chromatography using hexane-EtOAc  
30 (5:1) as an eluent to give the title products as a 1:1  
diastereomeric mixture. MS m/z (M+1)<sup>+</sup> 139.

3. 5-Bromo-1-ethyl-4-methyl-1H-imidazole-2-carbaldehyde and 4-bromo-1-ethyl-5-methyl-1H-imidazole-2-carbaldehyde

To a solution of 2.01 g (15 mmol) of 1-ethyl-4-methyl-1H-imidazole-2-carbaldehyde and 1-ethyl-5-methyl-1H-imidazole-2-carbaldehyde in 20 ml CH<sub>3</sub>CN is added 2.67 g (15 mmol) NBS. The mixture is stirred at room temperature for 4 hours and the solvent is evaporated in vacuo. The residue is dissolved in 35 ml of EtOAc and washed with water (3 x 15 ml) and brine (15 ml) then dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of the solvent provides a light yellow oil. Flash column chromatograph of the mixture on silica gel (4:1 hexane, EtOAc) provides the two isomers; both have MS *m/z* (M + 1)<sup>+</sup> 217.

4. 5-Bromo-1-ethyl-2-hydroxymethyl-4-methyl-1H-imidazole

To a stirred solution of 87 mg (0.4 mmol) 5-bromo-1-ethyl-4-methyl-1H-imidazole-2-carbaldehyde in 5 ml of MeOH is added 46 mg (1.2 mmol) of NaBH<sub>4</sub>. The mixture is stirred at room temperature for 3 hours and the solvent is removed in vacuo. The residue is dissolved in 15 ml of EtOAc and washed with 10 ml of water and 10 ml of brine. The organic phase is dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated. This clear oil is used in next step without further purification. MS *m/z* (M + 1)<sup>+</sup> 219.

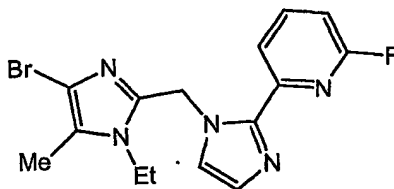
5. 5-Bromo-2-chloromethyl-1-ethyl-4-methyl-1H-imidazole hydrochloride

To a stirred solution of 85 mg (0.39 mmol) of 5-bromo-1-ethyl-2-hydroxymethyl-4-methyl-1H-imidazole in 4 ml of CH<sub>2</sub>Cl<sub>2</sub> is added 2 ml of SOCl<sub>2</sub>. The solution is stirred at room temperature for 4 hours, and the solvent is removed in vacuo. The residue is dissolved in 10 ml of CH<sub>2</sub>Cl<sub>2</sub>, then evaporated in vacuo. This is repeated twice to give 2-chloromethyl-1-ethyl-4-methyl-5-(3-trifluoromethyl-phenyl)-1H-imidazole hydrochloride as a light yellow foam which is used in next step without further purification. LC-MS (M+1) 237.

6. 2-[1-(5-Bromo-1-ethyl-4-methyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine (Compound 1)

To a solution of 74 mg (0.27 mmol) of 5-bromo-2-chloromethyl-1-ethyl-4-methyl-1H-imidazole hydrochloride in 1.5 ml of DMF is added 224 mg (1.62 mmol) of  $K_2CO_3$  and 45 mg (0.27 mmol) of 2-fluoro-6-(1H-imidazol-2-yl)-pyridine. The mixture is stirred at room temperature for 24 hours. 5 ml water is added and the mixture was extracted with EtOAc (3 x 15 ml). The combined extracts are washed with 15 ml brine, dried ( $Na_2SO_4$ ), filtered and evaporated in vacuo. The residue is purified by preparative TLC, developing with 10:0.4:0.05  $CH_2Cl_2$ -MeOH- $NH_4OH$  solution. The purified compound is then dissolved in 3 ml of EtOAc and to it is added 2 ml of 2N HCl in ether. The resulting suspension is stirred at room temperature for 15 minutes. Evaporation of the solvent provided the title compound as a white powder. LC-MS (M+1) 364.

B. 2-[1-(4-BROMO-1-ETHYL-5-METHYL-1H-IMIDAZOL-2-YLMETHYL)-1H-IMIDAZOL-2-YL]-6-FLUORO-PYRIDINE (Compound 2)



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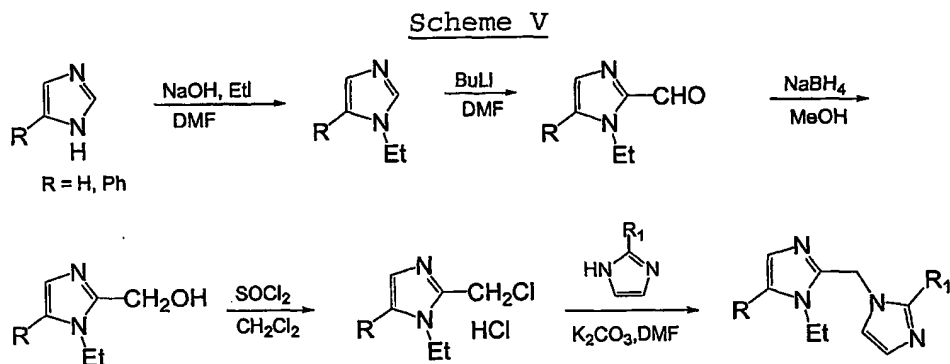
To a solution of 77 mg (0.27 mmol) of 4-bromo-2-chloromethyl-1-ethyl-5-methyl-1H-imidazole hydrochloride in 1.5 ml of DMF is added 224 mg (1.62 mmol) of  $K_2CO_3$  and 45 mg (0.27 mmol) of 2-fluoro-6-(1H-imidazol-2-yl)-pyridine. The mixture is stirred at room temperature for 24 hours. 5 ml water is added and the mixture was extracted with EtOAc (3 x 15 ml). The combined extracts are washed with 15 ml brine, dried ( $Na_2SO_4$ ), filtered and evaporated in vacuo. The residue is purified by preparative TLC, developing with 10:0.4:0.05

30

CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH solution. The purified compound is then dissolved in 3 ml of EtOAc and to it is added 2 ml of 2N HCl in ether. The resulting suspension is stirred at room temperature for 15 minutes. Evaporation of the solvent provides the title compound as a white powder. LC-MS (M+1) 364.

### C. 4-H, 4-PHENYL IMIDAZOLE COMPOUNDS

4-H and 4-phenyl-imidazole compounds are prepared essentially as described above, according to the following general scheme:



Representative compounds prepared by this method are shown in Table I.

Table I

Cmpd. No.	Compound	Name	MS (M+1)
5		2-[1-(1-ethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine	272
14		2-[1-(1-Ethyl-5-phenyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine	348

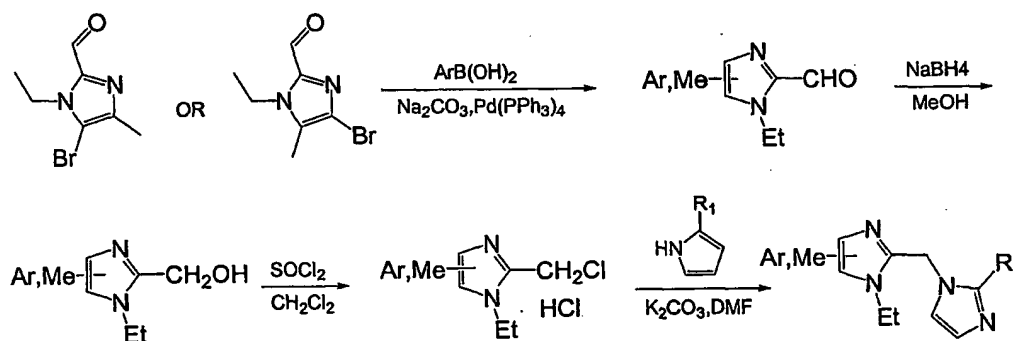
## EXAMPLE 2

Preparation of Representative Methyl Aryl- and Trifluoromethyl Aryl-Imidazole Compounds

5 This Example illustrates the preparation of representative methyl aryl- and trifluoromethyl aryl-imidazole compounds provided herein.

A. METHYL ARYL IMIDAZOLE COMPOUNDS

Methyl aryl imidazole compounds are prepared according to  
10 the following general scheme:

Scheme VI

1. 1-Ethyl-4-methyl-5-aryl-1H-imidazole-2-carbaldehyde or 1-Ethyl-5-methyl-4-aryl-1H-imidazole-2-carbaldehyde

15 To a solution of 0.217g (1 mmol) of 5-bromo-1-ethyl-4-methyl-1H-imidazole-2-carbaldehyde or 4-bromo-1-ethyl-5-methyl-1H-imidazole-2-carbaldehyde in 5 ml of toluene is added 0.116 g of Pd(PPh<sub>3</sub>)<sub>4</sub>, a solution of aryl boronic acid (2 mmol) in 4 ml of EtOH and 2 ml of 2M aqueous Na<sub>2</sub>CO<sub>3</sub> solution. N<sub>2</sub> is  
20 bubbled through the mixture for 20 minutes to degas the solution. The mixture is then refluxed under N<sub>2</sub> for 24 hours. After cooling, 10 ml water and 10 ml of EtOAc are added and the layers are separated. The aqueous layer is extracted with EtOAc (2 × 15ml) and the combined extracted are washed with  
25 brine (15 ml) then dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated in vacuo. The residue is separated by preparative TLC, developing with 10:0.4:0.05 CH<sub>2</sub>Cl<sub>2</sub>-MeOH-NH<sub>4</sub>OH solution.



2. 1-Ethyl-2-hydroxymethyl-4-methyl-5-aryl-1H-imidazole or 1-Ethyl-2-hydroxymethyl-5-methyl-4-aryl-1H-imidazole

To a stirred solution of (0.4 mmol) 1-ethyl-4-methyl-5-aryl-1H-imidazole-2-carbaldehyde or 1-ethyl-2-hydroxymethyl-5-methyl-4-aryl-1H-imidazole in 5 ml of MeOH is added 46 mg (1.2 mmol) of NaBH<sub>4</sub>. The mixture is stirred at room temperature for 3 hours and the solvent is removed in vacuo. The residue is dissolved in 15 ml of EtOAc and washed with 10 ml of water and 10 ml of brine. The organic phase is dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated. This clear oil is used in next step without further purification.

3. 2-Chloromethyl-1-ethyl-4-methyl-5-aryl-1H-imidazole hydrochloride or 2-Chloromethyl-1-ethyl-5-methyl-4-aryl-1H-imidazole hydrochloride

To a stirred solution of (0.39 mmol) of 1-ethyl-2-hydroxymethyl-4-methyl-5-aryl-1H-imidazole or 1-ethyl-2-hydroxymethyl-5-methyl-4-aryl-1H-imidazole in 4 ml of CH<sub>2</sub>Cl<sub>2</sub> is added 2 ml of SOCl<sub>2</sub>. The solution is stirred at room temperature for 4 hours, and the solvent is then removed in vacuo. The residue is dissolved in 10 ml of CH<sub>2</sub>Cl<sub>2</sub>, and then evaporated in vacuo. The resulting light yellow foam is used in next step without further purification.

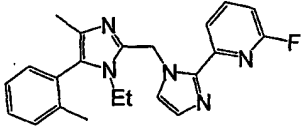
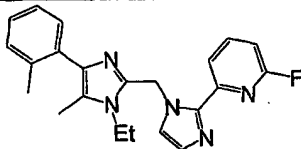
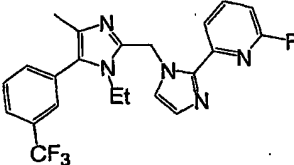
4. 2-[1-(5-Aryl-1-ethyl-4-methyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine or 2-[1-(4-Aryl-1-ethyl-5-methyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine

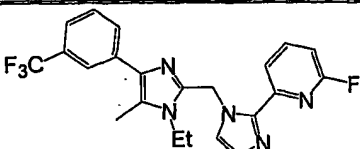
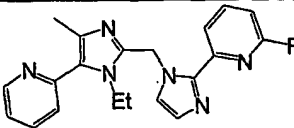
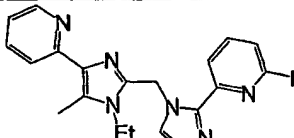
To a solution of (0.27 mmol) of 2-chloromethyl-1-ethyl-4-methyl-5-aryl-1H-imidazole hydrochloride or 2-chloromethyl-1-ethyl-5-methyl-4-aryl-1H-imidazole hydrochloride in 1.5 ml of DMF is added 224 mg (1.62 mmol) of K<sub>2</sub>CO<sub>3</sub> and 45 mg (0.27 mmol) of 2-fluoro-6-(1H-imidazol-2-yl)-pyridine. The mixture is stirred at room temperature for 24 hours. 5 ml of water is added and the mixture is extracted with EtOAc (3 x 15 ml).

The combined extracts are washed with 15 ml brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated in vacuo. The residue is purified by preparative TLC. The purified compound is then dissolved in 3 ml of EtOAc and to it is added 2 ml of 2N HCl in ether. The resulting suspension is stirred at room temperature for 15 minutes. Evaporation of the solvent provides the title compound hydrochloride salt as a white powder.

Representative compounds prepared by this method are shown in Table II.

Table II

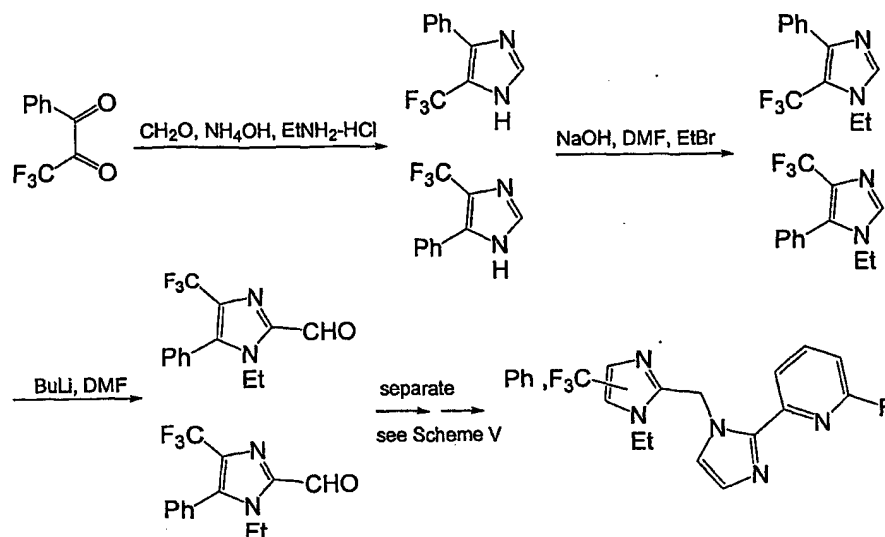
Cmpd. No.	Compound	Name	MS (M+1)
15		2-[1-(1-ethyl-4-methyl-5-o-tolyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine	376
16		2-[1-(1-ethyl-5-methyl-4-o-tolyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine	376
4		2-{1-[1-ethyl-4-methyl-5-(3-trifluoromethyl-phenyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine	430

Cmpd. No.	Compound	Name	MS (M+1)
3		2-{1-[1-ethyl-5-methyl-4-(3-trifluoromethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl}-6-fluoro-pyridine	430
12		2-{1-[1-ethyl-4-methyl-5-(2-pyridyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine	363
13		2-{1-[1-ethyl-5-methyl-4-(2-pyridyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine	363

#### B. TRIFLUOROMETHYL-ARYL-IMIDAZOLE COMPOUNDS

Trifluoromethyl-aryl-imidazole compounds are prepared according to the following general scheme:

Scheme VII



1. 4-Phenyl-5-trifluoromethyl-1H-imidazole and 5-Phenyl-4-trifluoromethyl-1H-imidazole

5        3,3,3-Trifluoro-1-phenyl-propane-1,2-dione (808 mg, 4 mmol), formaldehyde (37%, 0.34 ml, 4.4 mmol), ethylamine hydrochloride (1.07 g, 13.2 mmol) in concentrated  $\text{NH}_4\text{Cl}$  (6 ml) is refluxed for 3 hours. Water (15 ml) is added and the mixture is extracted with  $\text{EtOAc}$  (4 x 20 ml). The combined  
10        extracts are washed with water (20 ml) and brine (20 ml), then dried and evaporated. Flash column chromatography of the residue (10:0.4:0.05  $\text{CH}_2\text{Cl}_2$ - $\text{MeOH}$ - $\text{NH}_4\text{OH}$ ) provides a clear oil with LC-MS ( $\text{M}+1$ ) 213, which is the mixture of two isomers.

2. 1-Ethyl-4-phenyl-5-trifluoromethyl-1H-imidazole and 1-

15        ethyl-5-phenyl-4-trifluoromethyl-1H-imidazole

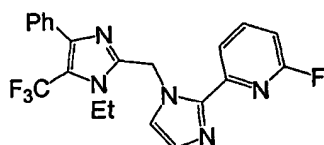
At  $0^\circ\text{C}$ , 0.8 g (20 mmol)  $\text{NaOH}$  is added to a solution of 4-phenyl-5-trifluoromethyl-1H-imidazole and 5-phenyl-4-trifluoromethyl-1H-imidazole (2.13 g, 10 mmol) in  $\text{DMF}$  (10 ml). The mixture is stirred at room temperature for 1 hour and  $\text{EtBr}$   
20        (0.75 ml, 12 mmol) is added. The mixture is stirred at room temperature overnight. The solvent is removed, water (25 ml) is added and the mixture is extracted with  $\text{EtOAc}$  (4 x 30 ml). The combined extracts are washed with brine (2 x 30 ml) and

dried. Evaporation of the solvent provides a clear oil. This oil is used directly, without further purification, in the next step. LC-MS (M+1) 241.

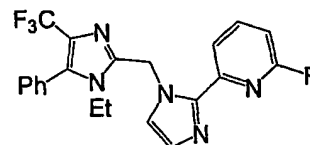
3. 1-Ethyl-4-phenyl-5-trifluoromethyl-1H-imidazole-2-  
 5 carbaldehyde and 1-Ethyl-5-phenyl-4-trifluoromethyl-1H-  
imidazole-2-carbaldehyde

A solution of 1-ethyl-4-phenyl-5-trifluoromethyl-1H-imidazole and 1-ethyl-5-phenyl-4-trifluoromethyl-1H-imidazole (2.4 g, 10 mmol) in 30 ml THF is chilled to -78°C, and 5 ml  
 10 (12.5 mmol) BuLi is added. The mixture is stirred at -78°C for 30 minutes, then DMF (3 ml) is added. The cooling bath is removed and the mixture is stirred at room temperature for 24 hours. The solvent is removed in vacuo and the residue is partitioned with 10 ml of water and 25 ml of EtOAc. The  
 15 aqueous layer is extracted with EtOAc (2 x 25 ml), and the combined extracts are washed with 20 ml of brine, then dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and evaporated in vacuo. The residue is purified by flash column chromatography using hexane-EtOAc (5:1) as an eluent. Both of the isomers have MS m/z (M+1)  
 20 269.

4. 2-[1-(1-Ethyl-5-phenyl-4-trifluoromethyl-1H-2-ylmethyl)-1H-  
imidazol-2-yl]-6-fluoro-pyridine (compound 17)  
and 2-[1-(1-Ethyl-4-phenyl-5-trifluoromethyl-1H-2-ylmethyl)-1H-  
imidazol-2-yl]-6-fluoro-pyridine (compound 18)



and



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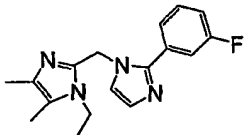
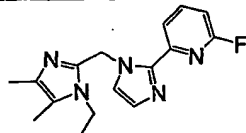
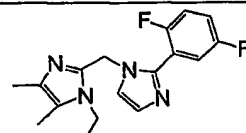
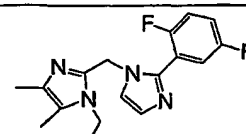
Conversion of 1-ethyl-4-phenyl-5-trifluoromethyl-1H-imidazole-2-carbaldehyde or 1-ethyl-5-phenyl-4-trifluoromethyl-1H-imidazole-2-carbaldehyde into 2-[1-(1-ethyl-5-phenyl-4-trifluoromethyl-1H-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine (compound 17) or 2-[1-(1-ethyl-4-phenyl-5-trifluoromethyl-1H-2-ylmethyl)-1H-imidazol-2-yl]-6-  
 30

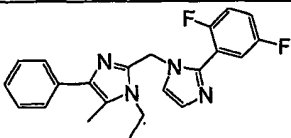
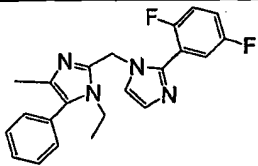
fluoro-pyridine (compound 18) is achieved essentially according to the procedure outlined in Scheme I and described in more detail in Example 1. LC-MS (M+1) 416.

### 5 C. ADDITIONAL COMPOUNDS

Additional representative compounds prepared according to Scheme I and the foregoing procedures are shown in Table III.

Table III

Cmpd. No.	Compound	<sup>1</sup> H NMR	Mass Spec (Cald./Obsd.M+1)
6		free base in CDCl <sub>3</sub> : 7.38-7.48, m (3H); 7.26, t (1H); 7.09, s (1H); 6.97 (s, 1H), 5.22, s (2H); 3.36, q, (2H); 2.13, s (3H); 2.05, s (3H); 0.77, t (3H)	298.36/299.30
7		free base in CDCl <sub>3</sub> : 8.17, dd (1H); 7.85 dd, (1H); 7.17, s (1H); 7.08, s (1H); 6.90, dd (1H); 6.00, s (2H); 3.97, q (2H); 2.18, s (3H); 2.12, s (3H); 1.02, t (3H)	299.35/300.30
8		free base in CDCl <sub>3</sub> : 7.22-7.37, m (1H); 7.18, m (2H); 7.16, s (1H); 6.97, s (1H); 5.05, s (2H); 3.43, q (2H); 2.15, s (3H); 2.04, s (3H)	316.35/317.40
9		free base in CDCl <sub>3</sub> : 7.26, m (1H); 7.15-7.19, m (2H); 7.12, s (1H); 6.96, s (1H); 5.06, s (2H); 3.17, t	330.38/331.20

Cmpd. No.	Compound	<sup>1</sup> H NMR	Mass Spec (Cald./Obsd.M+1)
		(2H); 2.12, s (3H); 2.05, s (3H); 1.21, m (2H); 0.69, t (3H)	
10		free base in CDCl <sub>3</sub> : 7.60, d (2H); 7.26-7.40, m (7H); 7.04, s (1H); 5.19, s (2H); 3.53, q (2H); 2.33, s (3H); 0.87, t (3H)	378.42/379.30
11		free base in CDCl <sub>3</sub> : 7.40, m (3H); 7.17-7.35, m (6H); 7.08, s (1H); 5.17, s (2H); 3.46, q (2H); 2.11, s (3H); 0.65, t (3H)	378.42/379.20

## EXAMPLE 3

Ligand Binding Assay

5        The high affinity of compounds of this invention for the benzodiazepine site of the GABA<sub>A</sub> receptor was confirmed using a binding assay essentially described by Thomas and Tallman (*J. Bio. Chem.* (1981) 156:9838-9842, and *J. Neurosci.* (1983) 3:433-440).

10       Rat cortical tissue was dissected and homogenized in 25 volumes (w/v) of Buffer A (0.05 M Tris HCl buffer, pH 7.4 at 4°C). The tissue homogenate was centrifuged in the cold (4°C) at 20,000 x g for 20 minutes. The supernatant was decanted, the pellet rehomogenized in the same volume of buffer, and  
15       centrifuged again at 20,000 x g. The supernatant of this centrifugation step was decanted and the pellet stored at -20°C overnight. The pellet was then thawed and resuspended in 25 volumes of Buffer A (original wt/vol), centrifuged at 20,000 x g and the supernatant decanted. This wash step was

repeated once. The pellet was finally resuspended in 50 volumes of Buffer A.

Incubations contained 100  $\mu$ l of tissue homogenate, 100  $\mu$ l of radioligand, (0.5 nM  $^3\text{H}$ -Ro15-1788 [ $^3\text{H}$ -Flumazenil], specific activity 80 Ci/mmol), and test compound or control (see below), and were brought to a total volume of 500  $\mu$ l with Buffer A. Incubations were carried for 30 min at 4°C and then rapidly filtered through Whatman GFB filters to separate free and bound ligand. Filters were washed twice with fresh Buffer A and counted in a liquid scintillation counter. Nonspecific binding (control) is determined by displacement of  $^3\text{H}$  Ro15-1788 with 10  $\mu\text{M}$  Diazepam (Research Biochemicals International, Natick, MA). Data were collected in triplicate, averaged, and percent inhibition of total specific binding (Total Specific Binding = Total - Nonspecific) was calculated for each compound.

A competition binding curve was obtained with up to 11 points spanning the compound concentration range from  $10^{-12}\text{M}$  to  $10^{-5}\text{M}$  obtained per curve by the method described above for determining percent inhibition.  $K_i$  values were calculated according the Cheng-Prusoff equation. Each of the compounds set forth above was tested in this fashion and each was found to have a  $K_i$  of  $< 1\mu\text{M}$ . Preferred compounds of the invention exhibit  $K_i$  values of less than 100 nM and more preferred compounds of the invention exhibit  $K_i$  values of less than 10 nM.

#### EXAMPLE 4

##### Electrophysiology

The following assay is used to determine if a compound of the invention acts as an agonist, an antagonist, or an inverse agonist at the benzodiazepine site of the  $\text{GABA}_A$  receptor.

Assays are carried out essentially as described in White and Gurley (NeuroReport 6:1313-1316, 1995) and White, Gurley,



Hartnett, Stirling, and Gregory (Receptors and Channels 3:1-5, 1995) with modifications. Electrophysiological recordings are carried out using the two electrode voltage-clamp technique at a membrane holding potential of -70 mV. *Xenopus laevis* oocytes are enzymatically isolated and injected with non-polyadenylated cRNA mixed in a ratio of 4:1:4 for  $\alpha$ ,  $\beta$  and  $\gamma$  subunits, respectively. Of the nine combinations of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits described in the White et al. publications, preferred combinations are  $\alpha_1\beta_2\gamma_2$ ,  $\alpha_2\beta_3\gamma_2$ ,  $\alpha_3\beta_3\gamma_2$ , and  $\alpha_5\beta_3\gamma_2$ . Preferably all of the subunit cRNAs in each combination are human clones or all are rat clones. The sequence of each of these cloned subunits is available from GENBANK, e.g., human  $\alpha_1$ , GENBANK accession no. X14766, human  $\alpha_2$ , GENBANK accession no. A28100; human  $\alpha_3$ , GENBANK accession no. A28102; human  $\alpha_5$ , GENBANK accession no. A28104; human  $\beta_2$ , GENBANK accession no. M82919; human  $\beta_3$ , GENBANK accession no. Z20136; human  $\gamma_2$ , GENBANK accession no. X15376; rat  $\alpha_1$ , GENBANK accession no. L08490, rat  $\alpha_2$ , GENBANK accession no. L08491; rat  $\alpha_3$ , GENBANK accession no. L08492; rat  $\alpha_5$ , GENBANK accession no. L08494; rat  $\beta_2$ , GENBANK accession no. X15467; rat  $\beta_3$ , GENBANK accession no. X15468; and rat  $\gamma_2$ , GENBANK accession no. L08497. For each subunit combination, sufficient message for each constituent subunit is injected to provide current amplitudes of >10 nA when 1  $\mu$ M GABA is applied.

Compounds are evaluated against a GABA concentration that evokes <10% of the maximal evocable GABA current (e.g., 1 $\mu$ M-9 $\mu$ M). Each oocyte is exposed to increasing concentrations of a compound being evaluated (test compound) in order to evaluate a concentration/effect relationship. Test compound efficacy is calculated as a percent-change in current amplitude:  $100 * ((I_c/I) - 1)$ , where  $I_c$  is the GABA evoked current amplitude observed in the presence of test compound and  $I$  is

the GABA evoked current amplitude observed in the absence of the test compound.

Specificity of a test compound for the benzodiazepine site is determined following completion of a concentration/effect curve. After washing the oocyte sufficiently to remove previously applied test compound, the oocyte is exposed to GABA + 1  $\mu$ M RO15-1788, followed by exposure to GABA + 1  $\mu$ M RO15-1788 + test compound. Percent change due to addition of compound is calculated as described above. Any percent change observed in the presence of RO15-1788 is subtracted from the percent changes in current amplitude observed in the absence of 1  $\mu$ M RO15-1788. These net values are used for the calculation of average efficacy and EC<sub>50</sub> values by standard methods. To evaluate average efficacy and EC<sub>50</sub> values, the concentration/effect data are averaged across cells and fit to the logistic equation.

#### EXAMPLE 5

##### MDCK Cytotoxicity Assay

This Example illustrates the evaluation of compound toxicity using a Madin Darby canine kidney (MDCK) cell cytotoxicity assay.

1  $\mu$ L of test compound is added to each well of a clear bottom 96-well plate (PACKARD, Meriden, CT) to give final concentration of compound in the assay of 10 micromolar, 100 micromolar or 200 micromolar. Solvent without test compound is added to control wells.

MDCK cells, ATCC no. CCL-34 (American Type Culture Collection, Manassas, VA), are maintained in sterile conditions following the instructions in the ATCC production information sheet. Confluent MDCK cells are trypsinized, harvested, and diluted to a concentration of  $0.1 \times 10^6$  cells/ml with warm (37°C) medium (VITACELL Minimum Essential Medium Eagle, ATCC catalog # 30-2003). 100  $\mu$ L of diluted cells is

added to each well, except for five standard curve control wells that contain 100  $\mu$ L of warm medium without cells. The plate is then incubated at 37°C under 95% O<sub>2</sub>, 5% CO<sub>2</sub> for 2 hours with constant shaking. After incubation, 50  $\mu$ L of  
5 mammalian cell lysis solution is added per well, the wells are covered with PACKARD TOPSEAL stickers, and plates are shaken at approximately 700 rpm on a suitable shaker for 2 minutes.

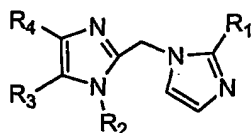
Compounds causing toxicity will decrease ATP production, relative to untreated cells. The PACKARD,  
10 (Meriden, CT) ATP-LITE-M Luminescent ATP detection kit, product no. 6016941, is generally used according to the manufacturer's instructions to measure ATP production in treated and untreated MDCK cells. PACKARD ATP LITE-M reagents are allowed to equilibrate to room temperature. Once  
15 equilibrated, the lyophilized substrate solution is reconstituted in 5.5 mL of substrate buffer solution (from kit). Lyophilized ATP standard solution is reconstituted in deionized water to give a 10 mM stock. For the five control wells, 10  $\mu$ L of serially diluted PACKARD standard is added to  
20 each of the standard curve control wells to yield a final concentration in each subsequent well of 200 nM, 100 nM, 50 nM, 25 nM and 12.5 nM. PACKARD substrate solution (50  $\mu$ L) is added to all wells, which are then covered, and the plates are shaken at approximately 700 rpm on a suitable shaker for 2  
25 minutes. A white PACKARD sticker is attached to the bottom of each plate and samples are dark adapted by wrapping plates in foil and placing in the dark for 10 minutes. Luminescence is then measured at 22°C using a luminescence counter (e.g., PACKARD TOPCOUNT Microplate Scintillation and Luminescence  
30 Counter or TECAN SPECTRAFLUOR PLUS), and ATP levels calculated from the standard curve. ATP levels in cells treated with test compound(s) are compared to the levels determined for untreated cells. Cells treated with 10  $\mu$ M of a preferred test compound exhibit ATP levels that are at least 80%, preferably

at least 90%, of the untreated cells. When a 100  $\mu$ M concentration of the test compound is used, cells treated with preferred test compounds exhibit ATP levels that are at least 50%, preferably at least 80%, of the ATP levels detected in  
5 untreated cells.

It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the spirit or scope of the present invention. Accordingly, the  
10 invention is not limited except as by the appended claims.

What is claimed is:

1. A compound of the formula:



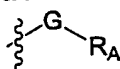
5 or a pharmaceutically acceptable salt thereof, wherein:

$R_1$  represents 5- to 10-membered aryl or heteroaryl, which is unsubstituted or substituted with from 1 to 4 groups independently selected from  $R_5$ ;

10  $R_2$  represents  $C_1$ - $C_8$ alkyl,  $C_2$ - $C_8$ alkenyl,  $C_2$ - $C_8$ alkynyl,  $C_3$ - $C_{10}$ cycloalkyl or  $(C_3$ - $C_{10}$ cycloalkyl) $C_1$ - $C_8$ alkyl, each of which is unsubstituted or substituted with from 1 to 3 substituents independently selected from  $R_5$ ;

$R_3$  and  $R_4$  are each independently selected from:

- 15 (a) hydrogen, halogen, nitro and cyano; and  
(b) groups of the formula:



wherein:

20 (i) G is a bond,  $C_1$ - $C_8$ alkylene, -NH-, -N( $R_B$ )-, -( $R_B$ )N-, O-, -C(=O)-, -C(=O)NH-, -C(=O)NR $_B$ -, -S(O) $_m$ -, -CH $_2$ C(=O)-, -S(O) $_m$ NH-, -S(O) $_m$ NR $_B$ -, -NHC(=O)-, -C(=NR $_B$ )-, HC=N-, -NR $_B$ C(=O)-, -NHS(O) $_m$ - or -NR $_B$ S(O)-; and

25 (ii)  $R_A$  and  $R_B$  are independently selected from  $C_1$ - $C_8$ alkyl,  $C_2$ - $C_8$ alkenyl,  $C_2$ - $C_8$ alkynyl and 3- to 12-membered carbocycles and heterocycles having 1 ring or 2 fused, pendant or spiro rings, each of which is unsubstituted or substituted with from 1 to 4 substituents independently selected from  $R_5$ ; and

(iii) m is 0, 1 or 2; and

30  $R_5$  is independently selected at each occurrence from halogen, hydroxy, nitro, cyano, amino,  $C_1$ - $C_8$ alkyl,  $C_1$ - $C_8$ alkoxy, mono- and di( $C_1$ - $C_8$ alkyl)amino,  $C_3$ - $C_{10}$ cycloalkyl, ( $C_3$ - $C_{10}$ cycloalkyl)alkyl, ( $C_3$ - $C_{10}$ cycloalkyl)alkoxy,  $C_2$ - $C_9$ heterocycloalkyl,  $C_1$ - $C_8$ alkenyl,  $C_1$ - $C_8$ alkynyl, halo( $C_1$ - $C_8$ )alkyl, halo( $C_1$ - $C_8$ )alkoxy, oxo, amino( $C_1$ - $C_8$ )alkyl and  
35 mono- and di( $C_1$ - $C_8$ alkyl)amino( $C_1$ - $C_8$ )alkyl.

2. A compound or salt according to claim 1, wherein R<sub>1</sub> is a 6-membered aromatic ring, unsubstituted or substituted with from 1 to 4 groups independently selected from R<sub>5</sub>.

5

3. A compound or salt according to claim 2, wherein R<sub>1</sub> is phenyl or pyridyl, unsubstituted or substituted with from 1 to 3 groups independently selected from halogen, C<sub>1</sub>-C<sub>6</sub>alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, C<sub>1</sub>-C<sub>6</sub>alkoxy and halo(C<sub>1</sub>-C<sub>6</sub>)alkoxy.

10

4. A compound or salt according to claim 3, wherein R<sub>1</sub> is substituted with one or two halogens.

5. A compound or salt according to claim 1, wherein R<sub>2</sub> is C<sub>1</sub>-C<sub>6</sub>alkyl or halo(C<sub>1</sub>-C<sub>6</sub>)alkyl.

15

6. A compound or salt according to claim 5, wherein R<sub>2</sub> is C<sub>1</sub>-C<sub>4</sub>alkyl.

7. A compound or salt according to claim 6, wherein R<sub>2</sub> is ethyl or propyl.

20

8. A compound or salt according to claim 1, wherein R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, halogen, alkyl, haloalkyl, and 5- to 7-membered aromatic carbocycles and heterocycles, wherein the carbocycles and heterocycles are unsubstituted or substituted with one or more of halogen, trifluoromethyl or methyl.

25

9. A compound or salt according to claim 8, wherein R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, halogen, trifluoromethyl, methyl, phenyl or pyridyl, wherein the phenyl or pyridyl is unsubstituted or substituted with halogen, trifluoromethyl or methyl.

30

35

10. A compound or salt according to claim 1, wherein: R<sub>1</sub> is phenyl or pyridyl, unsubstituted or substituted with from 1 to 3 groups independently selected from halogen, C<sub>1</sub>-

C<sub>6</sub>alkyl, halo(C<sub>1</sub>-C<sub>6</sub>)alkyl, C<sub>1</sub>-C<sub>6</sub>alkoxy and halo(C<sub>1</sub>-C<sub>6</sub>)alkoxy;

R<sub>2</sub> is C<sub>1</sub>-C<sub>4</sub>alkyl; and

5 R<sub>3</sub> and R<sub>4</sub> are independently hydrogen, halogen, trifluoromethyl, methyl, phenyl or pyridyl, wherein the phenyl or pyridyl is unsubstituted or substituted with halogen, trifluoromethyl or methyl.

10 11. A compound according to claim 1, wherein in an assay of GABA<sub>A</sub> receptor binding the compound exhibits an K<sub>i</sub> of 1 micromolar or less.

12. A compound according to claim 1, wherein in an assay of GABA<sub>A</sub> receptor binding the compound exhibits an K<sub>i</sub> of 100  
15 nanomolar or less.

13. A compound according to claim 1, wherein in an assay of GABA<sub>A</sub> receptor binding the compound exhibits an K<sub>i</sub> of 10  
20 nanomolar or less.

14. A compound according to claim 1, which is 2-[1-(5-Bromo-1-ethyl-4-methyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine.

25 15. A compound according to claim 1, which is 2-[1-(4-Bromo-1-ethyl-5-methyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine.

16. A compound according to claim 1, which is 2-{1-[1-Ethyl-5-methyl-4-(3-trifluoromethyl-phenyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine.  
30

17. A compound according to claim 1, which is 2-{1-[1-Ethyl-4-methyl-5-(3-trifluoromethyl-phenyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine.  
35

18. A compound according to claim 1, which is 2-[1-(1-Ethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine.
- 5 19. A compound according to claim 1, which is 2-[1-(1-Ethyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-3-fluoro-benzene.
- 10 20. A compound according to claim 1, which is 2-[1-(1-Ethyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-6-fluoro-pyridine.
- 15 21. A compound according to claim 1, which is 2-[1-(1-Ethyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene.
- 20 22. A compound according to claim 1, which is 2-[1-(1-Propyl-4,5-dimethyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene.
23. A compound according to claim 1, which is 2-[1-(1-Ethyl-5-methyl-4-phenyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene.
- 25 24. A compound according to claim 1, which is 2-[1-(1-Ethyl-4-methyl-5-phenyl-1H-imidazol-2-ylmethyl)-1H-imidazol-2-yl]-2,5-difluoro-benzene.
- 30 25. A compound according to claim 1, which is 2-{1-[1-Ethyl-4-methyl-5-(2-pyridyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine.
- 35 25. A compound according to claim 1, which is 2-{1-[1-Ethyl-5-methyl-4-(2-pyridyl)-1H-imidazol-2-ylmethyl]-1H-imidazol-2-yl}-6-fluoro-pyridine.



26. A pharmaceutical composition comprising a compound according to claim 1, in combination with a physiologically acceptable carrier or excipient.

5        27. The pharmaceutical composition of claim 26 wherein the pharmaceutical composition is formulated as an injectible fluid, an aerosol, a cream, a gel, a pill, a capsule, a syrup, or a transdermal patch.

10       28. A method for the treatment of anxiety, depression, a sleep disorder, attention deficit disorder, or Alzheimer's dementia, comprising administering to a patient in need of such treatment a therapeutically effective amount of a compound according to claim 1.

15       29. A method for potentiating a therapeutic effect of a CNS agent, comprising administering to a patient a CNS agent and a compound according to claim 1.

20       30. A method for improving short term memory in a patient, comprising the step of administering to a patient a therapeutically effective amount of a compound according to claim 1.

25       31. A method for determining the presence or absence of GABA<sub>A</sub> receptor in a sample, comprising the steps of:

- (a) contacting a sample with a compound according claim 1, under conditions that permit binding of the compound to GABA<sub>A</sub> receptor; and
- 30       (b) detecting a level of compound bound to GABA<sub>A</sub> receptor, and therefrom determining the presence or absence of GABA<sub>A</sub> receptor in the sample.

35       32. A method according to claim 31, wherein the compound is radiolabeled, and wherein the step of detection comprises the steps of:

- (i) separating unbound compound from bound compound; and

(ii) detecting the presence or absence of bound compound in the sample.

33. A method according to claim 32 wherein the presence  
5 or absence of bound compound is detected using autoradiography.

34. A method for altering the signal-transducing activity of GABA<sub>A</sub> receptor, comprising contacting a cell  
10 expressing GABA<sub>A</sub> receptor with a compound according to claim 1 in an amount sufficient to detectably alter the electrophysiology of the cell, and thereby altering GABA<sub>A</sub> receptor signal-transducing activity.

35. A method according to claim 34 wherein the cell  
15 recombinantly expresses a heterologous GABA<sub>A</sub> receptor, and wherein the alteration of the electrophysiology of the cell is detected by intracellular recording or patch clamp recording.

36. A method according to claim 34 wherein the cell is a  
20 neuronal cell that is contacted in vivo in an animal, the solution is a body fluid, and the alteration in the electrophysiology of the cell is detected as a change in the animal's behavior.

37. A method according to claim 36 wherein the animal is  
25 a human, the cell is a brain cell, and the fluid is cerebrospinal fluid.

38. A packaged pharmaceutical preparation comprising the  
30 pharmaceutical composition of claim 26 in a container and instructions for using the composition to treat a patient suffering from anxiety, depression, a sleep disorder, attention deficit disorder, Alzheimer's dementia or short-term  
35 memory loss.

39. The use of a compound according to claim 1 for the manufacture of a medicament for the treatment of anxiety,

depression, a sleep disorder, an attention deficit disorder, Alzheimer's dementia or short-term memory loss.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 03/13855

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D401/14 C07D403/14 C07D233/64 A61K31/4164 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, A	WO 02 060877 A (HOFFMANN LA ROCHE) 8 August 2002 (2002-08-08) abstract claims 1,3 examples	1-39
A	WO 02 02557 A (NEUROGEN CORP ;CAI GUOLIN (US); SHAW KENNETH (US)) 10 January 2002 (2002-01-10) abstract claims examples	1-39

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the International filing date
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- \*S\* document member of the same patent family

Date of the actual completion of the international search

7 August 2003

Date of mailing of the international search report

20/08/2003

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Authorized officer

Stix-Malaun, E

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 03/13855

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 28-37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

ation on patent family members

International Application No

PCT/US 03/13855

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02060877	A	08-08-2002	WO 02060877 A1	08-08-2002
			US 2002151715 A1	17-10-2002
WO 0202557	A	10-01-2002	AU 7029701 A	14-01-2002
			WO 0202557 A2	10-01-2002
			US 2002032200 A1	14-03-2002

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